EHRLICH II – 2nd World Conference on Magic Bullets

Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich

Nürnberg, Germany

October 3-5, 2008

Abstract Book
Combined targeting of growth-promoting genes with antisense oligonucleotides in human colorectal cancer cells: Chemo-sensitization potential

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Background: Aberrant expression of growth-promoting genes contributes to the growth advantage of tumor cells, targeting such genes with phosphorothioated antisense oligonucleotides, AS[5]ODNs, might therefore be useful in controlling the abnormal proliferation of cancer cells. To explore the potential of combination antigene therapy in human colorectal cancer cells, we have examined the in vitro effects of AS[5]ODNs targeting c- myb, c-myc and cdc2 in human colorectal cancer cell lines.

Method: Cancer cells were treated with c- myb-, c-myc- or cdc2-AS[5]ODNs individually or in combination. The effects of growth promoting-gene AS[5]ODNs on mRNA and protein expression were determined by RT-PCR and blot analysis. The effects of these combinations on cell growth, chemo-sensitization, apoptosis and genes controlling cell growth and apoptosis were monitored by MIT assay, DNA fragmentation and Real-time RT-PCR.

Results: mRNA and protein expression were dramatically reduced after treatment with c- myb-, c-myc-, or cdc2-AS[5]ODNs. Combined targeting of c- myb-, c-myc and cdc2 did had much higher dose and time dependent synergistic growth inhibitory effects, 5-100% and 5-95%, respectively, compared to single antigen therapy (5-50%). Combined targeting of c- myc / cdc2 also produced greater dose and time dependent additive or synergistic growth inhibitory effects (10-100%) compared to single antigen therapy (5-60%). The combined targeting of c- myb- c-myc / cdc2 exhibited much higher growth inhibitory effects (10-90%) compared to single antigen therapy (20-50%). Combined targeting of c- myb- / c-myc / cdc2 produced marked inducing effects on both apoptosis and chemo-sensitization to taxol, SF6, doxorubicin and vincristine. Real-time RT-PCR indicated down-regulation of mRNAs of cdk1, cyclin B1, cdc2, cyclin E1, cdck4, cyclin D1, Bcl2 and Bclcd, and up-regulation of mRNAs of p21, Bax and caspase.

Conclusion: Our study suggests that combination antigene therapy targeting c- myb-, c-myc and cdc2 can inhibit human colorectal cancer cell proliferation more effectively than monogene therapy by blocking the cell cycle and inducing apoptosis. Combination antigene therapy is thus a promising approach for cancer therapy.

Introduction of supercritical fluid extraction as a new sample-preparation procedure for isolation and identification of a pharmaceutical from biological fluids: Application to disposition kinetics

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Background: Since its commercial development in the early 1990s, supercritical fluid extraction (SFE) has attracted considerable attention as a sample-preparation procedure. However, other different sample preparation procedures, including precipitation, liquid and/or solid-phase extraction in biological fluids, also remain in use. Aims: In this investigation, SFE was introduced to isolate and identify orbifloxacin (OBFX) from plasma and milk of lactating does.

Methods: Four parameters, including the temperature and the pressure of supercritical fluid, modifier ratios, and dynamic extraction time, were evaluated for high-performance liquid chromatography coupled with fluorescence detector (HPLC-FLD). The linearity of the calibration curves as well as the instrument limit of detection /limit of quantitation (LOD/LOQ) were evaluated.

Results: The optimal conditions of the extraction process that yielded the maximum analyte extraction efficiencies were 150°C, 60°C, 250 kg/cm2, 30% vs. 35% methanol, and 40 min vs. 20 min, for plasma and milk, respectively. Good linearity (at least r2≥0.999) of the calibration curves was obtained over the range from 0.2 to 0.01 µg/mL. The method showed a good recovery rate (74.2–127.3%) and precision (expressed as relative standard deviations (RSDs) 1.64–20%). The instrumental LOD and LOQ values were 0.034 µg/mL vs. 0.004 µg/mL or 0.006 µg/mL vs. 0.02 µg L for plasma and milk, respectively. The method was successfully applied to estimate the pharmacokinetic variables of orbifloxacin in lactating does.

Conclusions: To the best of our knowledge, this is the first time that SFE has been applied to isolate an antimicrobial agent from biological fluids. This method is promising for clinical applications and for pharmacokinetic studies of various pharmaceuticals in biological fluids.

Misoprostol and Postpartum Hemorrhage

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It has been 100 years since Paul Ehrlich popularised the concept of a "magic bullet" during his 1908 Nobel prize lecture. Scientists have since keenly embarked on the search for these magic bullets. In the UK prostate cancer is the most common cancer in men diagnosed in men and the second leading cause of cancer death in men (1). Consequently a magic bullet is highly desired by patients and urologists alike.

Brachytherapy is ultrasound guided implantation of radioactive seeds into the prostate. Its popularity has increased as the use of transrectal ultrasound (TRUS) makes it easier to accurately direct the magic bullet. Patient selection is a key. In this scenario brachytherapy achieves a 12-year progression free survival of 96% (3).

Heat effects are attractive. HIFU is a bullet where prostate tissue is heated to the point of coagulative necrosis using high-energy ultrasound allowing the selective destruction of tissues. Long-term results from clinical trials are awaited. Freezing or cryotherapy of the prostate cancer has increased over the past few years, especially with the improvement in cryotechnology. Multiple hollow core probes are placed percutaneously under TRUS guidance. This causes cell destruction at 20 – 40°C, usually achieved by applying two cycles of freeze thaw. 96% of men with localised disease achieve a PSA of <0.2ng/ml within 6 months. Again long term results are awaited.

Finally, Docetaxel, chemotherapy agent, is a magic bullet that may be used to treat androgen-independent prostate cancer, with promising results (4).

1) http://info.cancerresearchuk.org/cancerstats/types/prostate/
2) Rapley H, Korth Lj, Elgamal AA et al Medim prost medine brachytherapy. Prostate specific antigen results in 219 patients with up to 12 years of observed follow-up (Cancer 2003; 89:125-41).
Effect Of Flunixin On Enrofloxacin Clearance And Steady-State Serum Concentrations In Calves

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Background: To evaluate whether the concomitant administration of flunixin may alter enrofloxacin pharmacokinetic parameters and the hepato-renal activities.

Methods: Ten clinically healthy, Frisian calves weighting 200-250 kg and 5-7 months of age were used. They were divided into three groups: first group was injected a single dose of enrofloxacin 2.5 mg/kg of body weight (BW) intravenously (IV). Second group was injected the same dose intramuscularly (IM). After 1 month washout period, the calves were divided into each group (n=5) and treated with enrofloxacin 2.2 mg/kg of BW one-hour prior to with the injection of enrofloxacin in a dose of 2.5 mg/kg BW. Controls were prepared with the first group IV and IM injection in the second group.

Results: Co-administration of flunixin with IV injection of enrofloxacin reduced the volume of distribution at steady state Vss and total body clearance (Clss) by 33% and 31%, respectively. After IM injection of enrofloxacin, the elimination half-life (t1/2a) and mean residence time (MRT) were shorter in the flunixin-medicated calves.

Conclusions: Concomitant administration of flunixin with enrofloxin induced significant alterations in pharmacokinetic parameters and hepato-renal function in calves. Therefore, concurrent administration of flunixin with enrofloxin should be avoided.
New Silver Compounds as Wound Healing Material Problems and Opportunities
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Wound care is a major medical problem affecting 10% of all hospitalized patients and costing more than $5 billion US$ per year in the USA alone. The major problem, especially with large wounds, such as burns, and chronic wounds includes slow healing. And that this not only means inconvenience, even today infected wounds may be lethal to hospitalized patients. The antibacterial properties of silver, and its low toxicity, combined with the ease of topical applications and surface coatings using the metal and its compounds has led to a revival of silver use in medicine. Silver containing wound dressings are today based on mainly silver or simple silver salts such as the nitrate or the sulphate. The only silver coordination compound used to any extent is the [Ag(sulfadiazine)]

In this contribution we will discuss some problems affecting different types of silver therapy, i.e. silver resistant bacteria (contrary to popular belief this does exist and may be a growing problem), coupling of silver and antibiotic resistance (remains to be proven, but a link exists), in vitro and in vivo studies (difficult to extrapolate and documented effect of existing preparations not always good). We will also discuss some entities that new coordination compounds may give in this respect and what grounds there are to expect that other species other than just the "naked" Ag ion could be the active antimicrobial agents. New silver coordination compounds were synthesized by direct mixing of aqueous solutions of silver nitrate and ethanolic solutions of the corresponding ligand. Also solid state techniques were employed to synthesis [Ag(α-amino-pyridine)3(NO3)]

Further synthesis and structural details are described elsewhere. UV- as well as H-NMR titrations were carried out to investigate the structural behavior and stability at different ratios. Antibacterial activity of the complexes was determined according to the recommendations of NCCLS (1999) for the use of a broth microdilution method. The activity (MIC) of some Ag(I) nitocinate and iso-nitocinate compounds compared to silver-sulfadiazine against some clinically isolated multi-resistant bacteria.

Central Nervous Systems involvement in dogs naturally infected by Leishmania chagasi/infantum
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Blood brain barrier (BBB) was described by Paul Ehrlich when he injected dyes in the blood stream and observed that all tissues stained, except the organs of central nervous system (CNS). This barrier is very efficient in controlling substances that could cause damage to the brain. However, several pathogens are able to cross it. In order to demonstrate that Leishmania chagasi/ infantum is also capable to produce lesions in CNS, the brain of thirty dogs naturally infected by the protozoon were studied. The samples were submitted to histopathological, immunohistochemical exams and Polimerase Chain Reaction (PCR). Histopathological analysis revealed that the amastigotes forms were associated to an inflammatory reaction. It was observed mainly in the cerebellum and thalamus. Both PCR and immunohistochemistry confirmed the presence of the parasite in 20% of the examined brains. Previous studies in our laboratory evidenced that, mice experimentally infected by L. amazonensis also developed inflammatory infiltrates composed by eosinophils and parasitized macrophages. Mast cells were frequently observed. Both studies demonstrated that Leishmania is able to change the permeability of BBB leading to lesions. Although, the brain is considered a healthy place immunologically privileged several pathogens can cross this barrier and cause damage. Recent developments have been postulated to explain how the efficient BBB is impaired. In accordance to Persidsky et al. (2009), it occurs during inflammatory process due to disruption of junction complexes between brain microvascular endothelial cells with subsequent formation of a paracellular route that facilitates entry of leukocytes into cerebral parenchyma. We suppose that Leishmania reached brain of mice and canine through immature monocytes.

PKC-ι inhibition by ICA-1 reduces cell proliferation in Neuroblastoma
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Abstract
Neuroblastomas are highly lethal tumors and 85% of cerebral neuroblastomas occur in children and 15% in adults. Neuroblastoma is the most common type of cancer in children. According to the American Cancer Society, there are approximately 6500 new cases of neuroblastoma each year in the United States. Despite significant educational efforts, improved diagnostic techniques, and rigorous therapies, neuroblastoma control remains static. To address this health issue, the objectives of this research was to investigate the use protein kinase C-ι (PKC-ι) inhibitors in the proliferation of neuroblastoma cells. Previous work has shown that inhibition of PKC-ι is a promising means by which to prevent and treat certain cancers. Here, we report the identification of a PKC-ι inhibitor (1H-imidazol-4-carboxamide, 5-amino-1-[2,3-dihydroxy-4-[(phosphonoxy) methyl] cyclopolymer]-[1R-[1,2,3-βι]-dihydroxy-4-[j-phosphonooxy)] methyl] cyclotetrol]-[1,2,3-βι]-[3,2 J, 3, 4 i, 0 j] (ICA-1) that targets a unique sequence (amino acid residues 469-475) in the catalytic domain of PKC-ι, inhibits PKC-ι activity and is effective in blocking the proliferation of BE(2)-C neuroblastoma cells. Our data support significant proof of concept that ICA-1 inhibits the proliferation of neuroblastoma cells and may be a novel chemotherapeutic for the treatment of patients with neuroblastoma.
Nicotinamide Mechanisms of Neuroprotection in Stroke

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Background: Stroke is a major cause of death in the USA. Although stroke can be treated by removing ischemic data or by administration of clot dissolving agents, therapy for stroke is inadequate. Patients frequently do not recover normal speech or motion following strokes. The current work explores the development of a new therapeutic agent, nicotinamide, for recovery from stroke.

Methods: The focal ischemia and reperfusion model of stroke in rats was used, as well as low-dose irradiation-induced DNA damage, while HR is important for repairing topoisomerase I-induced DNA damage. Intriguingly, NHEJ negatively affects survival of cells treated with the topoisomerase I poison camptothecin. We also find that loss of Artemis not only leads to increased camptothecin resistance, independently of Lig4, but also alleviates hypersensitivity of Lig4-null cells to topoisomerase II inhibitors.

Results: We find that NHEJ is critical for repairing topoisomerase II- as well as low-dose irradiation-induced DNA damage, while HR is important for repairing topoisomerase I-induced DNA damage. Intriguingly, NHEJ negatively affects survival of cells treated with the topoisomerase I poison camptothecin. We also find that loss of Artemis not only leads to increased camptothecin resistance, independently of Lig4, but also alleviates hypersensitivity of Lig4-null cells to topoisomerase II inhibitors.

Conclusions: 1) HR and NHEJ have different roles in the repair of topoisomerase-mediated DNA damage. 2) Our data have significant implications for chemotherapy involving topoisomerase inhibitors. 3) A series of human gene knockout cell lines are useful in assessing cellular DNA damage and repair induced by chemotherapeutic agents.

Prevention of Mucositis and Improvement in Compliance of Head & Neck Cancer Patients undergoing Radio-Chemotherapy by Curcumin

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Background: Oral mucositis (OM) is a common complication and a dose limiting toxicity in up to 90% of head & neck cancer patients (HNCP) undergoing radio-chemotherapy (RCT). Its adverse effects on schedule of therapy, quality of life, and economic burden to the patients and system warrant an urgent need for a potent cell specific radioprotector. Several adjunctive agents like folic acid, Vit-E, antibiotic mouth rinse etc have been tried to prevent OM without remarkable success. Curcumin has shown radioprotective as well as radiosensitizing potential in in vitro studies with its antioxidant, free radical scavenging activity and ability to arrest cell cycle in S2 and M phase in malignant cells. Objective of this pilot study was to evaluate effects of curcumin on OM in HNCP under going RCT.

Methods: 95 HNCPs were given conventional RCT and 109 HNCP were given 2 gm of curcumin/day orally in 3 divided doses in addition to RCT for two months starting from 3 days before planned radiation. OM gradation as per WHO oral toxicity scale has been tried to prevent OM without remarkable success. Curcumin has shown radioprotective as well as radiosensitizing potential in in vitro studies with its antioxidant, free radical scavenging activity and ability to arrest cell cycle in S2 and M phase in malignant cells. Objective of this pilot study was to evaluate effects of curcumin on OM in HNCP under going RCT.

Results: There was a significant decrease in incidence of grade III and IV OM (P < 0.001) among curcumin treated group. Patient compliance in terms of completion of scheduled RCT increased from 53% to 89.0% (P ≤ 0.001).

Conclusion: 1. Curcumin showed a significant adjudicate protective activity against RCT induced OM in HNCP. 2. Long term follow-up required for its effects on resilience and survival in HNCP.
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Background: Effective oral insulin delivery remains a challenge to the pharmaceutical industry. A novel formulation of insulin using new biomaterials is necessary to formulate and administer insulin via the oral route. Aims: 1) To develop novel biocompatible delivery materials through cross-linking for the delivery of insulin. 2) To study the level of protection of insulin against degradation when formulated using the new biomaterial. 3) To study the in vitro and in vivo release of the insulin and its effect on blood glucose level in diabetic rabbits.

Methods: Cross-linking of muin-sodium alginate of ratios 0.1 to 1.3 was effected in liquid paraffin using acetic at -30 °C. The microparticles were diffusion-filled and further coated with 1.5 % w/v cellulose acetate phthalate in acetone at -30 °C. Diabetes was induced in 32 rabbits weighing between 1.8-2.5 kg using 120 mg/kg of alloxan and these used for the anti-diabetic assessment. Insulin-loaded microparticles containing 50 IU/kg was given to the rabbits in 2 ml of distilled water using gastric intubation using distilled water as the negative control and 50 IU/kg subcutaneously as the positive control.

Results: The microparticles formed were generally multi-particulate, discrete and free flowing. Before insulin loading, microparticles were round and smooth, becoming fluffier, less spherical and larger with rough and pitted surface after insulin loading. The mean dissolution time of insulin from the microparticles prepared with sodium alginate, muin, sodium alginate: muin at ratios of 1:1, 3:1 and 1:3 were 1.21±0.75, 3.3±4.42, 6.6±6.23, 8.5±2.95 and 3.45±9.05 (min) respectively. The percentage blood glucose reduction for the subcutaneously administered insulin was significantly (p<0.001) higher than for the formulations. The blood glucose reduction effect produced by the orally administered insulin-loaded microparticles prepared with 3 parts of sodium alginate and 1 part of muin after 5 h was, however, equal to that produced by the subcutaneously administered insulin solution.

Conclusion: This study shows that the oral route could be an effective alternative for the delivery of insulin using this polymer cross-link.

TOPOLOGICAL IMMUNOTHERAPY WITH DIPHENYLICYCLOPROPENONE IN VITILIGO: A PRELIMINARY EXPERIENCE

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Background: Despite recent significant therapeutic advances, vitiligo remains as a clinical conundrum. Topical immunotherapy has been extensively tested in the treatment of various dermatologic disorders especially those believed to have an immunologic basis. The aim of this study was to evaluate the role of topical DPCP in the treatment of vitiligo.

Methods: Nineteen patients with limited vitiligo lesions were enrolled in this study. After sensitization with 2% DPCP, progressively higher concentrations beginning at 0.001% were applied weekly for 6 months to depigmented skin. The maximum concentration of DPCP in acetone was 2%.

Results: Thirteen of 19 patients were evaluated at the end of 6 months. Four patients with focal vitiligo, 3 patients with vitiligo vulgaris, and 3 patients with segmental vitiligo showed marked (grade 3) repigmentation. More marginal and central repigmentation with hyperpigmented borders was seen in the majority of lesions. Further controlled trials should be undertaken to evaluate the use of topical DPCP in vitiligo, as this preliminary study is encouraging.

Studies on Interactions of Water Soluble Vitamins with Zinc Through cell, Protein and Animal Models in Health and Disease

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Background: Zinc (Zn) is required as a catalytic, structural and regulatory ion for enzymes, proteins and transcription factors and is thus a key trace element in many homeostatic mechanisms of the body. Vitamins like riboflavin, nicotinic acid, thiamine, pantothenic acid and ascorbic acid have functional groups capable of forming complexes with Zn. However, the interaction of water-soluble vitamins with Zn has not received much attention.

Methods: We have examined the Zn-vitamin interactions at a variety of conditions like different Zn concentrations, different cell and protein models and under normal and oxidative stress (OS) conditions. The interactions were studied in vitro, by using erythrocytes under deficient, normal and excess Zn states. Results: Under Zn-deficient state, thiamine significantly enhanced the erythrocyte Zn uptakes (p<0.05), whereas ascorbic acid and riboflavin inhibited it (p<0.05). In another study, an in vitro erythrocyte Zn uptake was compared among healthy and diabetic subjects and it was found that Zn uptakes of healthy subjects were 17-52% higher than those for diabetic subjects. Furthermore, erythrocyte super oxide dismutase, plasma ascorbic acid and status of riboflavin were negatively correlated with Zn uptakes in healthy subjects (p<0.01). These interactions were also studied in precision cut rat liver slices, where it was found that folic acid showed inhibitory effect on Zn uptake under both normal and OS conditions as seen by dose response curves. Ascorbic acid showed marked enhancing effect on Zn uptake under OS. These in vitro interactions were confirmed in vivo using male Wistar rats. The 21 days old rats were used to examine the effect of niacin supplementation on Zn absorption under chronic OS generated by test-buty hydro peroxide at a dose of 0.2 mm/kg body weight on Zn absorption and improved antioxidant enzyme profile. The albumin being the major Zn carrier protein in plasma and the albumin bound Zn (ABZn) comprises 80% plasma Zn. Folic acid and thiamine significantly enhanced the ABZn (p<0.01), while nicotinic acid inhibited Zn binding to albumin.

Conclusions: These results collectively suggest that vitamins are playing an important role in distribution of circulating Zn among albumin, blood cells and liver and giving a new dimension to their functionality in Zn metabolism in health and disease conditions.
Association of Fluoroquinolone and ESBL-Resistance in Gram-Negative Organisms from Oncology Patients of Lagos University Teaching Hospital (LUTH), Nigeria

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Background: This study determined the gram-negative bacilli associated with various cancer infections and defined fluoroquinolone susceptibility and Extended-Spectrum Beta-Lactamase production in isolated strains.

Methods: Materials for research were blood culture, urine, aspirates, fluids and swabs from cancer wounds. Samples were cultured and organisms isolated were determined using API system (Bio-Merieux). Antimicrobial resistance was evaluated by the disc diffusion method according to NCCLS/CLSI recommendations and ESBL detection was carried out using the Double Disk Synergy Test method.

Results: Of the 104 strains isolated 22 (21.4%) were found to be resistant to only ciprofloxacin. Only 1 of these resistances to ciprofloxacin was observed to have an accompanying production of ESBL. Of the 7 isolates that resistance to a combination of two fluoroquinolones, 2 (28.6%) were found to be ESBL-producers. Cross resistance to the 3 quinolones tested, occurred in 40 (38.8%) of the strains isolated. The strains in this group were observed to be associated in most of the cases with MDR [35 (37.5%)] and production of ESBL [16(41%)]. This group was observed to be predominant amongst strains of E. coli, Pseudomonas spp and Klebsiella spp.

Conclusion: Cross-resistance to fluoroquinolones has emerged amongst these clinical isolates and more worrisome is its association with ESBL-Production and Multidrug Resistance. Multi-drug resistance may be one of the contributing factors to the high mortality rate amongst these group of patients reported in developing countries. Antibiotic resistance surveillance is thus of utmost importance in contributing to the reduction of the high morbidity and mortality rate reported amongst cancer patients in this part of the world.

Effect of Diabetes Mellitus on Pharmacokinetics (PK) and Pharmacodynamics (PD) of Immunosuppressive agents: Ciclosporin, Tacrolimus and Mycophenolic Acid

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Background: A large percentage of kidney transplant recipients are diabetics but little is known on the effect of diabetes on the disposition or effect of immunosuppressant drugs. This study compared the pharmacokinetic and pharmacodynamic parameters of ciclosporin or tacrolimus and mycophenolic acid in diabetic (D) and non-diabetic (ND) patients.

Methods: The PK and PD studies included 42 and 32 stable kidney transplants, sampled for 12 or 2 hours post dose, respectively. Approximately 50% of patients were diabetic (D) that were demographically matched with non-diabetic (ND) controls. Immunosuppressive agents’ total, unbound and metabolite concentrations were measured by LC/MS/MS. Markers of T-cell activity (ATP concentration in CD4 cells, intracellular cytokines IL-2, IFN-γ and TNF-α in CD3 cells) and B-cell activity (co-stimulatory proteins CD54 [ICAM-1], CD86 [B7.2] and CD95 [Fas antigen] in stimulated CD19) were determined. Inosine monophosphate dehydrogenase (IMPDH) activity was also measured in some patients.

Results: Pharmacokinetics: As summarized in the Table, both ciclosporin (CSA) AUC0—∞ and AUC were reduced in Ds and CAa free fraction was higher. Tacrolimus absorption was delayed with no effect on the overall AUC. Diabetes delayed mycophenolate moefetil (MMF) absorption rate but not the enteric coated Na-MPA. The concentration of some metabolites of ciclosporin or MPA was lower in Ds.

Conclusions: Diabetes variability affect the PK/PD of immunosuppressive agents. Adjusting immunosuppressive dose guided by the PD markers may prove useful in dose individualization of immunosuppressive agents in diabetics.

Chemotherapy of advanced colorectal carcinoma under hemodialysis

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Background: Recent progress of hemodialysis therapy (HD) has prolonged the life span of patients suffering from end stage renal disease (ESRD) and it increased the complication of malignant tumors. It is reported that the prevalence of colorectal cancer in HD patients is higher than that in a control population. Saltz regimen; combination therapy of irinotecan, 5-fluorouracil, and leucovorin is widely used for the treatment of metastatic colorectal cancer after the large randomized phase III trials. Chemotherapy of HD patients is less clear. There are few pharmacokinetic data of most cytotoxic agents much less combination chemotherapy. We encountered a patient of colorectal cancer on HD. It was decided to give combination chemotherapy with monitoring the pharmacokinetics of the patient.

Methods: CPT-11 was administered just after HD at a dose of 50 mg/m² by 90-min intravenous infusion; followed by i.v. 10 mg/m², administered over the course of 15min; and 5-FU (400 mg/m²), given by bolus intravenous injection after i.v. Three drugs were given on days 1, 8 and 15 of a 35-day cycle. Blood samples were collected before administration and at 0.5, 4, 12, 24 hours after administration. Plasma concentration of CPT-11, SN-38 and 5-FU were compared to those in reference control values without ESRD. The plasma concentration of CPT-11, SN-38 and 5-FU were analyzed using high performance-liquid chromatography (HPLC).

Results: The pharmacokinetic results for each post administration value were not statistically different compared with the data of normal renal function. As for side effects, he experienced grade III hematological toxicity, which was easily controlled with G-CSF.

Conclusions: These data suggest that Saltz regimen can be feasible for colorectal cancer patients receiving HD without dose reducing.
Retargeting anticancer drugs to drug resistant cancers by using polymer Biotransport technology. Clinical proof of the concept

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Background: Anthracyclines are amongst the most widely used anticancer agents in man, but are limited by toxicity considerations, as well as by inherent or induced drug resistance. We have recently discovered that nonionic block copolymer surfactants, i.e. hydrophobic polyethylene oxide polypropylene oxide block copolymers (Pluronics) can considerably reduce drug resistance of various tumour cell lines to anthracyclines and other cytotoxic drugs. Following this finding, we have developed a Pluronic-based formulation of doxorubicin (SP1049C) that is thermodynamically stable, safe and provides doxorubicin with high efficacy against both drug resistant and drug sensitive tumours.

Methods: Safety and plasma pharmacokinetics of SP1049C were evaluated in phase I clinical trial that was carried out in 26 advanced cancer patients; and efficacy of the product was tested in phase II clinical trial, in 21 patients with metastatic adenocarcinoma of the esophagus.

Results: The results of phase I trial demonstrated comparable safety and PK profiles of SP1049C to that of doxorubicin. The phase II trial revealed that the product has a high anticancer activity. The overall response rate to SP1049C was 47%, (95% CI: 24-71), and median survival was 10 months, which compares favourably with other most active single agents tested in this indication.

Conclusions: The performed clinical studies have assured a further clinical development of SP1049C. At present it is entering an expanded international clinical phase III efficacy program including a single 300 patient pivotal phase III trial in patients with upper gastrointestinal tract.

Novel reporter probes for HSV1-tk gene expression

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Herpes simplex virus type-1 thymidine kinase (HSV1-tk) gene is being used as a suicide gene for gene therapy of cancer. Cancer cells are transduced with a retroviral vector carrying HSV1-tk gene. The gene expressed in the proliferating cells produces an enzyme(s) which can transform ganciclovir into a triphosphate form by cellular kinases. The ganciclovir tri-phosphate becomes a prodrug and inhibits DNA polymerase, thus kills the tumor cells.

Methods: Numerous reporter gene constructs have been developed during the past decade, and these are primarily purine and pyrimidine nucleoside analogues, such as F-FIAU, F-FHBG, F-FEAU, and F-FEAV have been recognized as ideal probes for PET imaging of HSV1-tk gene expression. We have studied these probes in the animal models and demonstrated that both F-FHBG and F-FEAV have efficacy for imaging of HSV1-tk gene expression, and they can be used selectively in PET imaging of native and mutated HSV1-tk gene expression. Thus F-FEAU is suitable for PET imaging of the native HSV1-tk gene expression; and F-FHBG is suitable for PET imaging of mutated HSV1-tk gene expression. This presentation will focus on these novel probes used for PET imaging of HSV1-tk gene expression with an emphasis on their application in pre-clinical animal models and human studies.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts

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Drug-Drug and Drug-Biomolecule Photoinduced Reactions in Preparations for Topical Use

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Background: There is a growing concern for the adverse effects caused by exposure to indoor/outdoor light after assumption of drugs. The first concern is obviously for topical preparations. The rather sparse literature on photo toxicity rarely examines the chemical reactions underlying to the biological effect. In general, there is a feeling that oxygen activation is the main mechanism, but the example below show that this is not always the case.

Methods: In vitro studies were carried out by irradiating aequous solution of the drugs under indoor/outdoor light at a measured flux. The photoproducts were isolated and the structure determined. The explanation was repeated in the presence of heterocycles mimicking the behaviour of nucleotides. Time-resolved studies for the detection of chemical intermediates were also performed. These studies and the structure of the products allowed to propose viable mechanisms, here concerning some UV filters used in sunscreens as well as some bactericides with eye-toxicity.

Results: Dibenzoxyethanes (DBM, in the utameric form under the applicable conditions) are a virtually constant component of commercial sunscreens in view of the high absorption in the UV-A. These products are quite photostable but are often associated with cyaninates in the preparations, because the latter absorbs the UV-B. However, if the combination of the two filters is exposed to light, both are rapidly consumed via a photocycloaddition reaction. Furthermore, irradiated DMBs photostacks to a variety of molecules present in the skin, e.g. to fats. As a result, the skin itself appears to be dually light-sensitive, with light in the eye drops, such as lomotein (LOM), and to ascorbic acid, known to damage the skin to the eye. In both cases a fast photodecomposed decomposion takes place, with liberation of active oxygen forms and formation of an aggressive intermediate (an arylation) that adds to a various compounds, including electron-rich heterocycles considered as a model of nucleotides, but not to water.

This negative effect that exposure to light should be minimized (e.g. LOM has been found to be genotoxic), but also suggest that flavinolized heterocycles may be considered for a novel type of photodynamic therapy based on the selective addtion to biomolecules by photoproduced arylation cations, veritable magic bullets.

Abstracts Page A-8

Protective Effect of Dimebolin on the TNF-Alpha-Induced Lipid Disorders in Mice Brain

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Background: Dimebon (Dimebolin) is an antiglutamic disease which has been used clinically in Russia since 1983. Recently Dimebolin has attracted renewed interest after being shown to have positive effects on persons suffering from Alzheimer’s disease. Animal studies have shown that dimebon operates through multiple mechanisms of action, both blocking the action of neurotoxic beta-amyloid proteins and inhibiting L-type calcium channels, modulating the action of AMPA and NMDA glutamate receptors, and may exert a neuroprotective effect by blocking cytokine signals induced by proinflammatory cytokines such as TNF-alpha which are believed to play a central role in Alzheimer’s disease. Inflammatory response induced by TNF-alpha suggests that this cytokine affects the phospholipid metabolism and subsequent production of eicosanoids, ceramide, and ROS that may potentiate brain injury.

Methods: This study included 65 male mice (weight: 20 ± 2 g, age: ± 3 SD). TNF-alpha (10mg per mice), dimebon (0,2 mg/kg ) and their combination were injected to mice interperitonealy. Changes in levels of phospholipid molecular species (phosphatidylcholine, lysophosphatidylcholine, phosphatidyl ethanolamine, sphingomyelin) and galactosylceramide in hippocampus, cerebellum and cerebral cortex within 30 min, 2, 4 and 24 hours after injection were detected by chromatogaphy-mass-spectrometry.

Results: Maximal changes in phospholipids and galactosylceramides contents of different molecular species after single TNF-alpha administration were found in the hippocampus, and decrease in phospholipids and galactosylceramide after 24 hours. Dimebon itself did not induce changes in lipid species in brain sections, but protected lipids against disorders induced by TNF-alpha in mice brain.

Conclusions: Modern strategies in the search of new therapeutic approaches are expected to be based on the multitarget profile of new drugs. According to our results TNF-alpha may serve as a new target for dimebon. Dimebon preventing lipids disorders in brain induced by TNF-alpha might have a positive anti-inflammatory effects, preventing the negative response of nerve cells to the pathological process.

The Role of Cell Membrane Lipid Environment in Antigenic Peptide Structure-function

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Background: The cell membranes control the extra- and intracellular exchange. The lipid structure, their organization in the membrane and around membrane proteins but also their dynamics in the two dimensions of the membrane are all essential characteristics of each cell type, for their selective role in the cell traffic. The aim of our study was to address the role of the lipid on an HIV-1 vaccine epitope structure and on the interaction with target cell membranes : either CD4+ T-cells or epithelial cells.

Methods: Biophysical, biochemical and biological methods have been used. Peptides derived from HIV-1 envelope glycoprotein gp41 were chemically synthesized or produced as recombinant peptides. To study lipid dynamics and domain organization due to protein interaction, liposomes of different lipid composition, mimicking either the viral membrane or target cell membranes were chemically obtained and fluorescence resonance energy transfer (FRET) has been used. Nuclear Magnetic Resonance (NMR) allowed defining peptide solution structure in presence of micelles. Binding of known HIV-1 antibodies to the epitopes inserted into liposomes were measured by enzyme linked immunosassays (ELISA).

Results: Different peptides from conserved regions of the HIV-1 envelope glycoprotein gp41 have been studied, including: 1) P1, the minimal Membrane Proximal Region (MPR) that permits interaction with mucosal galactosylceramide HIV-receptor and contains epitopes recognized by gp41-specific, broadly neutralizing IgGs, 2) P5 and 2) P5 P1 extended at its N-terminal by a calcium binding site. The alpha-helical structure of the peptides on DPC micelles is conserved or is induced, depending on the lipid composition, thereby enabling P1 to move laterally within liposome bilayer. The Kds of both 2SF and 4E10 IgGs measured on viral liposome and infectious virus are similar in the nM range and much lower than for the binding of the free P1 peptide. For P5, the role of calcium on its structure and its antiviral properties when in interaction with lipids was shown essential.

Conclusion: Thus, the defined lipid environment of MPER-derived peptides and environmental conditions (pH, calcium, ...) appear as essential for their structure and therefore for the design of an immunogen inducing broadly neutralizing antibodies to HIV-1, and also microbicides.

Protective effects of Nigella sativa extract and its components against chromium VI-induced toxicity in Nile tilapia (Oreochromis niloticus) and zebrafish (Danio rerio)

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Background: Chromium is an increasing health concern for aquatic environments, however, the mechanism of chromium toxicity in aquatic species is yet unknown. Hexavalent chromium VI (CrVI) is the dominant toxicant at some superfund sites within Egypt.

Methods: The aim of the current study was to evaluate the genotoxicity potential of hexavalent chromium VI (CrVI) in Nile tilapia (Oreochromis niloticus) and zebrafish (Danio rerio) using semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR), and possible protective effects of N. sativa for 30 days.

Results: The semi-quantitative RT-PCR results indicated that treatment with Cr(VI) at 4.37 and 1.75 mg/l for tilapia and zebrafish, respectively resulted in a significant increase in hepatic and brain mRNA level of cytochrome 450 gene family including CYP1A2, CYP3A and CYP2E1 in both fish species compared to control group. Moreover, Cr(VI) was found to induce severe histological changes in liver, brain and gills of the tested fish. On the other hand, the combined treatment showed that mRNA level of CYP1A2, CYP3A and CYP2E1 decreased significantly in the groups treated with Cr(VI) plus N. sativa oil or thymoquinone compared to the groups treated with the crude extract or Cr(VI) alone accompanied with a significant improvement in the histological picture of the liver, brain and gills. However, N. sativa was found to be more effective.

Conclusion: It could be concluded that N. sativa is a promising candidate against CrVI damage resulted from the exposure to different environmental pollutants.
Abstract

Silver nanoparticles have been known to have inhibitory and bactericidal effects. Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and is a major health problem. The combination effects of silver nanoparticles with the antibacterial activity of antibiotics have not been studied. Here, we report on the synthesis of metallic nanoparticles of silver using a reduction of aqueous Ag+ ion with the culture supernatants of Klebsiella pneumonia. Also in this investigation, these nanoparticles were evaluated for their part in increasing the antimicrobial activities of different antibiotics against Staphylococcus aureus and Escherichia coli. The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin were increased in the presence of silver nanoparticles against both test strains. The highest enhancing effects were observed for vancomycin, amoxicillin and penicillin G against Staphylococcus aureus.

Results:

The average mean diameters of gliclazide beads decreased with decreasing polymer concentration, increasing speed of stirring, and increasing the internal phase volume. All prepared beads possessed excellent fluidity. The swelling behaviour was strongly dependent on polymer concentration in the formulation and the pH of the medium. The in vitro release experiments revealed that the swelling is the main parameter controlling the release rate of gliclazide from the beads. In vivo studies on diabetic rabbits showed that the hyperglycaemic effect induced by gliclazide loaded alginate beads was significantly greater and more prolonged than that induced by the marketed conventional gliclazide tablet (Gliclazide 8). Results: The average mean diameters of gliclazide beads decreased with decreasing polymer concentration, increasing speed of stirring, and increasing the internal phase volume. All prepared beads possessed excellent fluidity. The swelling behaviour was strongly dependent on polymer concentration in the formulation and the pH of the medium. The in vitro release experiments revealed that the swelling is the main parameter controlling the release rate of gliclazide from the beads. In vivo studies on diabetic rabbits showed that the hyperglycaemic effect induced by gliclazide loaded alginate beads was significantly greater and more prolonged than that induced by the marketed conventional gliclazide tablet (Gliclazide 8).

Conclusions: Alginate beads can control, improve and prolong the systemic absorption of the gliclazide through their mucoadhesive properties. This effect results in maintaining tight blood glucose level and improved patient compliance.

Abstract

Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli

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Abstract

Silver nanoparticles have been known to have inhibitory and bactericidal effects. Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and is a major health problem. The combination effects of silver nanoparticles with the antibacterial activity of antibiotics have not been studied. Here, we report on the synthesis of metallic nanoparticles of silver using a reduction of aqueous Ag+ ion with the culture supernatants of Klebsiella pneumonia. Also in this investigation, these nanoparticles were evaluated for their part in increasing the antimicrobial activities of different antibiotics against Staphylococcus aureus and Escherichia coli. The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin were increased in the presence of silver nanoparticles against both test strains. The highest enhancing effects were observed for vancomycin, amoxicillin and penicillin G against Staphylococcus aureus.

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Conclusions: Alginate beads can control, improve and prolong the systemic absorption of the gliclazide through their mucoadhesive properties. This effect results in maintaining tight blood glucose level and improved patient compliance.
Clinical Pharmacokinetics of Gentamicin: Estimation of Initial Dosing Parameters in Hospitalized Patients at Kuwait Hospital.

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Background: Gentamicin has a narrow range between its therapeutic and toxic blood levels. This has prompted the development and wide use of pharmacokinetic dosing equations in order to maximize drug safety and efficacy. Dosing equations commonly rely on estimating gentamicin clearance (ClG) and volume of distribution (VdG). These parameters are subject to considerable variability’s. The objective of this study was to: (a) develop equations for estimating ClG and VdG based on Kuwaiti population and (b) evaluate these equations by comparison with other methods in their predictive ability to estimate ClG and VdG.

Methods: ClG and VdG were calculated in 47 patients (group 1) using the Sawchuk-Zaske method. Regression analysis was used to derive a correlation between creatinine clearance (ClCr) and ClG, VdG and actual body weight (ABW). Based on actual ClCr and VdG values, the predictive ability of the estimated parameters from the regression equations was validated and compared with 4 methods, using mean error (ME) (bias), mean squared error and root mean squared error (MSE and RMSE, respectively). All equations were also evaluated in an independent second group (group 2) of 23 patients.

Results: (a) The mean (SD) for ClG and VdG was 4.0 (±1.8) L·h\(^{-1}\) and 16.6 (±6.7) L, respectively. (b) The derived equations were: ClG (ClCr°1.117 (r = 0.791) and VdG (0.160/ABW)\(\times\)6.004. (r = 0.952). In comparison to the four published methods, the derived equations were found to be less biased (ME<0.00), and more precise (MSE<16.8, RMSE<5.02) in predicting ClG (p<0.05), and less biased (ME=0.01) with no difference in precision (MSE=36.22, RMSE=4.59) in predicting VdG (p=0.05). This precision was confirmed in the second group of 23 patients, where the derived equations were less biased (ME<0.1) and more precise (MSE<3.22, RMSE=1.48) in predicting ClG (p<0.05) and no differences were found for prediction of VdG (p=0.05).

Conclusion: The equations developed in this study provided a reliable estimation of ClG and VdG. It is planned to use them at Kuwait Hospitals help provide individualized patient dosing information to physicians.


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Background: Changes in the morphology and function of the chondrocytes during the Osteoarthritis (OA) progression can be related with the expression of molecules involved in the inflammatory process. Some of these molecules could be involved in synovial fluid composition, to stop the progression of OA. Our objectives were: 1) To describe the chondrocytes phenotype during early OA. 2) To identify possible target molecules involved in phenotypical changes in OA chondrocytes. 3) To identify programmed cell death of OA chondrocytes.

Methods: The experimentally OA-induced model was accomplished by unilateral knee meniscectomy and post-surgery training; normal rats were used as a control. The right femoral condyles were removed and processed for either electron microscopy (EM) or immunohistochemistry (IHQ). Samples from rats with 1 to 5 days were analyzed. Samples from OA rats were analyzed at 20 and 45 td.

Results: The OA knee showed a reduction of the chondrocytes’ nuclear area, and a disorganization of the extracellular matrix (ECM). The matrix components were reduced and the chondrocytes were in contact with the ECM. Vimentin cytoskeleton was also decreased. IL-1β expression was absent and IL-10 was constant in all samples. Finally the cell death analysis showed a co-expression of autophagic and apoptotic mechanisms.

Conclusion: Our results suggest that for early OA, chondrocytes changes its phenotype in order to synthesize proteins required for extracellular matrix (ECM) repairment. However, when its capacity is overwhelmed, chondrocytes begin the synthesis of catabolic molecules like IL-1β and TNF-α that stimulate an inflammatory process. Furthermore, the decrease of anti-inflammatory molecules could be involved in the beginning of the OA. Finally, the chondrocytes execute its own process of cell death, that include both autophagy and apoptosis.

Complexation of Itraconazole with Cycloextrins for Enhanced Solubility, Dissolution and Bioavailability

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Background: Itraconazole is an anti fungal agent whose poor aqueous solubility restricts its use for the treatment of oropharyngeal candidiasis, which is the first symptom of HIV infection. Therefore, the aim of this study was to prepare Itraconazole-cycloextrin inclusion complexes in solid state by different methods in order to enhance the solubility, dissolution and bioavailability of itraconazole.

Methods: The formation of inclusion complexes between Itraconazole and α-, β- and hydroxypropyl-β-cycloextrin (HP-β-CD) were assessed using phase solubility techniques. Solid state inclusion complexes between Itraconazole and cycloextrins were prepared using supercritical (SC) CO\(_2\) and conventional methods. The physico-chemical properties of the products were characterized by UV, DSC, FTIR, PXRD and SEM. Dissolution amounts of the products obtained by different methods were measured in gastric fluid. Finally, pharmacokinetic studies of the inclusion complexes were conducted in blood, liver and kidney of male Wistar rats to assess the bioavailability of the prepared complexes.

Results: The aqueous solubility of itraconazole increased linearly as a function of cycloextrin concentration according to the rank order: HP-β-CD > β-CD > α-CD. Inclusion formation was influenced by the preparation technique. Products obtained by the SC CO\(_2\) method were among the ones showing the highest interaction between Itraconazole and the CDs, leading to about three times higher dissolution amounts than pure itraconazole. Temperature, pressure and drug:CD ratio had significant effect on the inclusion yield. In vivo drug pharmacokinetic studies showed that the Itraconazole-β-CD product prepared using SC CO\(_2\) results in higher solubility of itraconazole than those obtained by physical mixing or coprecipitation methods.

Conclusions: Cycloextrins significantly improved the solubility of Itraconazole in aqueous solutions, which would improve the therapeutic effects of Itraconazole against antifungal infections. SC CO\(_2\) method proved to be an effective technique for preparing solid complexes between cycloextrins and Itraconazole. Since SC CO\(_2\) method as an exotic solvent residue, products obtained by this method should provide minimal side effects in humans.

Molecular Dynamics Simulations of human membrane transport proteins from the Major Facilitator Superfamily.

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The Major Facilitator Superfamily (MFS) constitutes the largest group of secondary active transporter proteins, with members ubiquitous in all kingdoms of life. MFS members transport many different substrates, from ions and amino acids to nucleotides and lipids. MFS proteins transport substrates by forming channels through the membrane. The core of the MFS transporter is a transmembrane protein containing 11-17 α-helices per monomer. Each α-helix is arranged as a β-barrel, with the single exposed face of each β-barrel representing the substrates binding site. The closed structures are conserved in all organisms, indicating a common evolutionary origin. The transmembrane domains are highly conserved, with a conserved motif known as the Walker A and Walker B sequences. This conserved motif is responsible for ATP binding and hydrolysis. The MFS superfamily is divided into several subfamilies based on sequence similarity and functional characteristics. The most well-studied subfamily is the ABC-transporters, which transport substrates against a concentration gradient.

Our results suggest that for early OA, chondrocytes changes its phenotype in order to synthesize proteins required for extracellular matrix (ECM) repairment. However, when its capacity is overwhelmed, chondrocytes begin the synthesis of catabolic molecules like IL-1β and TNF-α that stimulate an inflammatory process. Furthermore, the decrease of anti-inflammatory molecules could be involved in the beginning of the OA. Finally, the chondrocytes execute its own process of cell death, that include both autophagy and apoptosis.
Verapamil Reverts Acute Renal Functional Impairment Induced by Angiotensin II Converting Enzyme Inhibitors

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Background: Angiotensin II converting enzyme inhibitors have been effective in arresting glomerulosclerosis. Initially, we thought that this would not occur exclusively to the benefit of glomerulosclerosis but, in any case, it has become evident that, at least in part, the non-renal action of these agents is as important a component of their beneficial effects. Among these, particularly relevant is the effect of preventing and/or reversing renal dysfunction associated with an increase in renal intraglomerular pressure. Verapamil has been described as being capable of decreasing renal intraglomerular pressure, with an attenuation of the maladaptive responses of the tubuloglomerular feedback system. In this preliminary study, we have shown that the combination of verapamil and an ACE-I not only prevents kidney injury but also reverses the effects of the ACE-I on kidney function.

Methods: We have compared the effects of enalapril (10 mg/day) and enalapril plus verapamil (10 mg/day). Glomerular filtration rate (GFR) was measured by a clearance of inulin and blood pressure by radio-telemetric methods. We also measured plasma creatinine and albuminuria.

Results: The results show that the combination of enalapril plus verapamil is more effective than enalapril alone in preventing the increase in creatinine and albuminuria induced by the ACE-I. These results suggest that the combination of enalapril plus verapamil is more effective than enalapril alone in preventing the increase in creatinine and albuminuria induced by the ACE-I.

Conclusions: The combination of enalapril plus verapamil is more effective than enalapril alone in preventing the increase in creatinine and albuminuria induced by the ACE-I.
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**Background:** Hepatitis B Virus (HBV) is a unique DNA virus which replicates through an RNA intermediate, lacks proof reading ability, has high viral replication rate. This leads to random mutations with amino acid substitution in reverse transcriptase region. In clinical parlance antiviral drug resistance is defined as selection of variants bearing amino acid substitution confirming reduced susceptibility to drug those results in primary or secondary treatment failure. For the management of HBV infection in addition to interferon/PEG-Interferon/Thymosin alpha 4, oral antiviral like lamivudine, adefovir, entecavir, Telbivudine are licensed and Tenofovir and entecavir are licensed for HIV & HBV coinfection. Success rate of these antiviral agents do not exceed more than 30% to 40% in long term treatment and with prolonged therapy, menace of antiviral resistance exists. Clinical consequence of resistance is decreased HBsAg clearance, reverse of histological improvement, increased rate of disease progression, clinical decompensation or even death in patients with cirrhosis, risk of graft loss and death in liver transplant recipients, transmission of drug resistance strain and vaccine failure mutation.

**Methods:** We analyzed real life data on our patients receiving long term Lamivudine treatment and development of resistance clinical consequences and their management...

**Results:** Our study included 82 patients (male 66, Age range 5-85 years). Of the 82 patients 50 patients were HBsAg +ve. These patients received mean duration of Lamivudine therapy for 3.24 years. The study included 50 (34%) patients who developed resistance to Lamivudine, 32 patients were HBsAg –ve. Mean duration of treatment with Lamivudine was 2.8 years (2-12 years). 22 out of 32 patients showed Lamivudine resistance. The presentation of Lamivudine resistance was clinical decompensation 3, serum-hepatitis 7, flare of liver enzymes 8, and increased viral load 5. All the patients who were receiving resistance were treated with addition of Adefovir to Lamivudine. Mean period of 8.5 months of follow up; 2 patients died due to decompensation, remaining patients are stable with normalized liver function

**Conclusion:** Antiviral drug resistance is a major problem in management of chronic HBV infection. Combining second drug with no cross resistance at appropriate time seem to be best policy currently.

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**Pharmacokinetic study of rivastigmine in Iranian healthy subjects following 3 and 4.5 mg dosing using a simple and sensitive HPLC-UV method**

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**Background:** Rivastigmine is relatively new drug and the evaluation of its pharmacokinetic properties in different ethnic populations is important to optimize the choice of dosage regimen. For the pharmacokinetic study of rivastigmine, a simple and rapid but also a highly sensitive and selective bioanalytical assay method should be developed and validated for the determination of rivastigmine in human plasma. The assay was used for pharmacokinetic study in Iranian healthy subjects 3) To compare the pharmacokinetics of rivastigmine following 3 and 4.5 mg dosing.

**Methods:** A simple and reproducible HPLC method with spectrophotometric detection of rivastigmine was developed and validated for the determination of rivastigmine in human plasma. The assay was used for pharmacokinetic study of rivastigmine capsules in healthy Iranian subjects following 3 and 4.5 mg dosing. 23 healthy Iranian volunteers participated in both groups. Food and drinks were not allowed until 3 h after ingestion of the capsules. Multiple blood samples (5 ml) were collected and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 8 h post-dosing. A non-compartmental analysis was used in the data processing.

**Results:** In HPLC analysis, it was founded that in addition to reversed-phase retention, other retention mechanisms such as hydrogen-binding or ion-exchange are probably involved in the chromatographic behaviour of rivastigmine. Thiophenyl should not be used in the mobile phase, while an acetonitrile mobile phase and chromatography at high temperatures can increase theoretical plates for rivastigmine. A selective extraction of rivastigmine from plasma was obtained using butanol: methanol (8:2, v/v) and back extraction into diluted acetic acid. The newly developed HPLC-UV method had an limit of quantification (LOQ) of 0.5 ng/ml, which is comparable to LOQ of 0.2 ng/ml obtained by current LCMS methods. The pharmacokinetic studies showed that rivastigmine has a rapid oral absorption with a large inter-subjects variations. The mean values of maximum plasma concentration (Cmax) (in the range under the pharmacokinetic concentration-time curve from time 0 to 8 hours (AUCt)), were 8.27 ng/ml and plasma half-life following administration of the rivastigmine at 3 mg dosing were 6.92 h, 0.98 h, 11.95 ng/ml, 12.78 ng/ml and 1.11 h, respectively, and for 4.5 mg dosing 10.74 ng/ml, 0.91 h, 21.60 ng/ml, 22.98 ng/ml and 1.22 h, respectively.

**Conclusions:** For the first time, a highly sensitive HPLC-UV method was developed and validated for rivastigmine assay in plasma. Pharmacokinetics of rivastigmine in Iranian healthy subjects were comparable with results obtained in other ethnic populations. As reported by others, the oral bioavailability of rivastigmine increased with dose.

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**Antibiotic resistance: what can we learn from evolution?**

**AMINOV BI**

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**Background:** The brilliant ‘Zauberkrugel’ (Magic bullets) idea of Paul Ehrlich was a major breakthrough in treating infectious diseases. It opened the whole new era in medicine and led to the discovery of many antibiotics that saved millions of lives. Despite the considerable success, however, bacteria invented various strategies to overcome these drugs thus compromising the magic power of antibiotics. The number of pathogens that learned how to dodge these bullets is increasing and the question arises of how they acquired these properties? Aim: reconstruction of evolutionary history of selected antibiotic resistance genes to answer this question.

**Methods:** Four sets of genes encoding resistance to tetracyclines, macrolides, lincosamides, streptogramins, and vancomycin and fluorquinolones were chosen for this analysis: 1) tetracycline resistance genes, encoding ribosomal protection proteins; 2) the erm genes encoding enzymes that methylate the specific adenine residue in the 23S rRNA molecule; 3) the vanHAX; and 4) the qnr genes conferring resistance to the fluoroquinolones. The sequences were downloaded from GenBank and aligned using ClustalX ver. 1.83. Maximum-likelihood and Bayesian inference were used to reveal the evolutionary history of these genes.

**Results:** Phylogenetic reconstruction suggested a long evolutionary history of diversification of antibiotic resistance genes that began well before the ‘antibiotic era’. There is no indication that lateral gene transfer from antibiotic-producing bacteria has played any significant role in shaping the pool of antibiotic resistance genes in pathogenic and commensal bacteria. The primary antibiotic resistance gene pool originated and diversified within the environmental bacterial communities, from which the genes were mobilized and penetrated into taxonomically and ecologically distant bacterial populations, including pathogens.

**Conclusions:** Enormous metabolic diversity of bacteria allows them to come up with protection mechanisms even against novel antibiotics. To preserve the magic of novel antibiotic bullets we have to pay more attention to the pool of antibiotic resistance genes in the environment and carefully monitor the possible movement of such genes into commensal and pathogenic bacteria.
Activity of Antimalarial Constituents of Spathodea campanulata

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Background: The search for antimalaria drugs is a continuous one because of the devastating effect of the disease. Aim: To determine the antimalarial properties of Spathodea campanulata. Methods: This study included 165 Swiss albino mice in Fink and Kretschmar’s, and Rane in vivo tests. Five mice were used per treatment, weight range: 18-22g. In the Fink and Kretschmar’s test, each mouse was inoculated with Plasmodium berghei berghei and treated post-infection subcutaneously once daily for 4 days with plant constituents, chloroquine or blank control. The % parasitemia was evaluated on the fifth day post-infection. In the Rane test, the mice were treated with the drugs once daily for 4 days starting 3 days post-infection. The % parasitemia for each mouse was determined for 5 days starting from the fourth day post-infection. The active constituents of the plants were isolated by column chromatography and characterized.

Results: Antimalarial principles of stem bark were ursolic acid, tomentosolic acid, 20S,20-hydroxysteroidal acid and caffeic acid from leaves. In Fink and Kretschmar’s test ursolic acid at 15-60 mg/kg/day produced 34-97% suppression of parasitemia and mean survival period of 13-25 days. Tomentosolic acid at 10-80 mg/kg/day produced 35-82% suppression of parasitemia and mean survival period of 10-19 days. 20S,20-hydroxysteroidal acid at 20-80mg/kg/day produced 11-53% suppression of parasitemia and mean survival period of 8-15 days. The aqueous leaf extract at 50-400 mg/kg/day produced 0-74% suppression of parasitemia. Chloroquine at 10 mg/kg/day produced 98% suppression of parasitemia and mean survival period of 28 days. In Rane test the aqueous leaf extract at 50-400 mg/kg/day produced mean survival period of 10-16 days. Ursolic acid at 15-45 mg/kg/day produced mean survival period of 9-24 days. 20S,20-hydroxysteroidal acid at 20-80 mg/kg/day produced mean survival period of 9-16 days. Tomentosolic acid at 5-40 mg/kg/day produced mean survival period of 9-18 days. Blank control gave mean survival period of 7 days in both tests.

Conclusions: 1) The antimalarial principles of Spathodea campanulata demonstrated significant schizontocidal properties, the activity of chloroquine was however superior to any of them. 2) The activity of the antimalarial principles provides the scientific basis for the use of Spathodea campanulata in the management of malaria in traditional medicine.

Metalloantibiotics: Synthesis and Antibacterial Activity of Metal(II) Complexes Containing Cephalosporin and Sulfathiazole

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Background: The interaction of antibiotics with main and transition metal ions has attracted our attention and compelled us to combine their chemical in order to improve the stability and efficiency of antibiotics. Thus, designing a new area of research in the synthesis of stable metalloantibiotic compounds that may be used as active drugs working effectively against antibiotic resistance species. Methods: The metal(II) complexes were prepared by mixing clear solutions of the appropriate cephalosporin sodium salt (1 mmol) and NiCl2.HO or CuCl2 metal salts (1 mmol) in distilled water (10 mL) and sulfathiazole (1 mmol) in EtOH (10 mL). The reaction mixture was then stirred at room temperature for 12 h. and green precipitates formed. The precipitated complexes were filtered off, washed with water, MeOH and ether and dried under reduced pressure at room temperature. Yield 55-65%. No attempts to use different molar ratios to prepare the complexes were made. Antibacterial activities were tested using the paper disc diffusion method. The chosen strains were Staphylococcus aureus ATCC 29523, Escherichia coli ATCC 11775, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumonia ATCC 23357, Salmonella enteritidis CDC 64 and Bacillus subtilis ATCC 6551.

Results: Nickel(II) and copper(II) react with cephalosporins plus sulfathiazole (Htz) to form the following mixed ligand complexes: [M(celazolin)(stz)(H2O)], [M(cephalothin)(stz)(H2O)], [M(cefazolin)(stz)], [M(cephalexine)(Htz)] and [M(cephalexine)(stz)(Cl)] (Htzazolin = ceftazolin, Htzcephalin = cephalothin, Htzofloxin = cefofloxin) which were characterized by x-ray diffraction and spectroscopic methods. Their spectra indicate that most of the cephalosporins are acting as a monoaonic multideterminate chelating agent, the exception being ceftriaxone which is dianionic. The complexes are acting with the solvents and probably have polymeric structures. They have been screened for antibacterial activity in DSM5 solutions against several bacteria, and the results are compared with the activity of cephalosporins. The [M(cephalexine)(stz)(Cl)] complexes showed better activity than cefotaxime against a battery strains, including P. aeruginosa and S. aureus where cefotaxime is inactive. Conclusions: The synthesized compounds showed antibacterial properties. In comparison, the copper(II) and nickel(II) complexes containing ceftriaxone plus sulfathiazole showed better activity against several bacterial strains than the cefotaxime, thus introducing a novel class of metal-based bacterial agents.

Production, isolation, partial characterization and antimicrobial spectrum of a novel bacteriocin produced by a Lactobacillus plantarum strain in fermentation

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Background: Fermentation broths (in MRS) of an isolated Lactobacillus plantarum strain exhibited strong antimicrobial activity against common food spoilage and also pathogenic bacteria. Antimicrobial activity was measured in the agar diffusion assay using a total of 9 indicator food-grade bacteria. The antimicrobial activity appeared to be higher against closely related species and Lactobacillus curvatus was chosen as the most suitable among the tested microorganisms to serve as indicator for the quantification of the produced bacteriocin. The exhibited antimicrobial activity was due to the production of a proteinaceous compound, a bacteriocin, most of which appears to be cell-associated.

Methods: A method of mechanical and physicochemical treatments were applied to washed cells in attempting to solubilize the bacteriocin. The most convenient method for extraction was centrifugation at 20200 rcf for 10 minutes at 4°C. Repeated tricine – SDS – PAGE electrophoresis of samples taken at various fermentation time-points showed that bonds as ~ 30kDa were of interest. The position was finally determined by overlayering the gel with MRS agar in which L. curvatus was embedded.

Results: The M.W. of the bacteriocin was estimated at 30kDa. The isolated bacteriocin lost its activity after treatment with lipase and -chymotrypsin, but retained activity following proteolytic treatment. The stability of bacteriocin was studied over a range of pH, T and mechanical stresses.

Conclusions: It appeared to be heat labile, a characteristic indicative, along with M.W. and the sensitivity to lipase and -chymotrypsin, of a certain category of antimicrobial peptides, the class IV of bacteriocins produced by lactic acid bacteria. Fermentation kinetics studies performed in a stirred tank bioreactor showed that the production of the bacteriocin was not associated to growth, but it was rather formed as a secondary metabolite.

Systems-directed targeted therapies in metastatic tumors: Equitable to reductionist therapy approaches?

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Introduction: As we consider the exchange of information between tumor, adjacent stroma cells, and cells of the involved organ from a systems perspective, we may disregard operational constraints that a combination of activities triggered by specific action systems must be intended by single participating pathophysiological mechanisms, such as inflammation, angiogenesis, Warburg effect, immune response, extracellular matrix remodeling, proliferation, apoptosis, coagulation. Activities that seem to be operationally induced by the action systems, perspectives of an enhancement of complexity. We hypothesized, that tumor systems-directed therapies might have the capability to use aggregated action effects, as adjustable sizes to therapeutically modulate the tumor systems stability, homeostasis, and robustness.

Methods: We performed a retrospective analysis of recently published data on 278 patients with advanced and heavily pre-treated (10% to 63%) vascular sarcoma, melanoma, renal clear cell, cholangiocellular, mucopidermoid, and hepatocellular carcinoma, hormone-refractory prostate cancer, glioblastoma, and multifocal Langerhans’ cell histiocytosis enrolled in nine multi-center phase II trials (13 centers). Each patient received a multi-targeted systems-directed therapy that consisted of metronomic low-dose chemotherapy, a COX-2 inhibitor, combined with one or two transcription modulators, pioglitazone +/- dexamethasone or IFN-alpha.

Results: These treatment schedules may attenuate the metastatic potential, angiogenesis-associated inflammation, may even site-specifically and in a long-term disease stabilization followed by prolonged objective response (3% to 48%) despite poor monotherapy of the respective drugs. Progression-free survival (PFS) data are comparable with those of reductionist-designed standard first-line therapies targeting preferably tumor cell-specific pathways.

Conclusions: Differential response patterns indicate the therapies’ systems biological activity. Structured systems-directed therapies in metastatic cancer, targeting amongst others inflammation and neoangiogenesis, may break the barrier of complexity of tumor-stroma-interactions, and get a source for detecting toxicities of tumor-associated aggregated action effects as adjustable sizes for targeted biomodulatory therapies. Biomodulation of systems biological processes facilitates comparatively high efficacy at moderate toxicity.
A diarylquinoline targeting the energy supply of M. tuberculosis

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We discovered a diarylquinoline (TMC207 or R207910) with potent bactericidal activity against drug-sensitive and drug-resistant Mycobacterium tuberculosis. Whole genome sequencing of resistant mutants suggested that the drug targets the energy supply of mycobacteria by inhibition of the ATP synthase. The oligomeric subunit $c$ (AtpE) of ATP synthase was validated as the target by genetic, biochemical and binding assays. Unlike other TB drugs, TMC207 is equally active against growing and dormant TB bacilli, making it a good candidate for shortening TB therapy.

In mice, four weeks of TMC207 monotherapy exceeds the bactericidal activities of isoniazid and rifampin by at least 1 log unit. Substitution of rifampin, isoniazid, or pyrazinamide (the World Health Organization's first-line treatment regimen) with TMC207 accelerated bactericidal activity, leading to complete culture conversion after 2 months of treatment in some combinations, against 5 months for the standard regimen. Four months of treatment with rifampin + pyrazinamide + TMC207 yielded the same relapse rate as six months of the standard regimen. Similar improvements were observed when TMC207 was combined with drugs to treat MDR-TB, suggesting that use of TMC207 may also significantly reduce the duration of treatment of MDR-TB.

The bactericidal activity of TMC207 was confirmed in patients in a one week early bactericidal activity trial and the drug is now being investigated in a phase 2 trial in MDR TB patients

Preoperative use of Analgesia in Appendicitis

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Background: Appendicitis is a common cause of acute abdominal pain, early anaesthesia is considered previously as it could mask physical signs and hence delay the diagnosis and surgical intervention, now this has been challenged

Methods: A prospective, experimental study had been carried out in Ibn Sinna General Hospital from November 2008 to March 2007, our aim was to determine the influence of Diclofenac sodium DSvoltaren) on masking the diagnosis of acute appendicitis.

The data collected by using well designed questionnaire with observation during the period of admission before the operation.

Results: The study includes 80 patients (40 as cases and 40 as controls) and The result revealed that most of the symptoms (fever, anorexia, nausea and vomiting) and signs (tenderness, obturator and Psoas signs, local guarding and rigidity), in addition to the rate of perforation and the vital sign were not hidden by DSvoltaren) with P value >0.05,while other symptoms (pain) and signs (rebound tenderness, Rovsing and pointing) had been hindered by the use of DSvoltaren). The most common presenting symptom in placebo and DS group was pain (100%) which showed a marked decrease in severity in those who received voltaren as analgesia (72.2%).

Conclusion: Some of the symptoms and signs of acute appendicitis were masked by the use of analgesia, while others were not and overall Diclofenac sodium did not influence the decision of diagnosis or the management of acute appendicitis.
Single Chain Antibodies (ScFv)s and Immunogen conjugates: Computational and Functional Approaches

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Background: ScFvS in which the variable heavy and light chains are connected by a peptide linker maintain the binding specificity and affinity of the parental antibody IgG. ScFv coupled to highly toxic molecules (immunogen conjugates) are currently being developed for cancer therapy. Aims: 1) To assess the effects of specific mutations on the stability, structure and dynamics of the scFv antigen binding site. 2) To develop an in silico procedure for evaluating physicochemical properties of two tumor-targeting anti-HER2 immunogen conjugates. 3) To propose plant-based expression systems for high-level scFv production.

Methods: This study included four scFvS (scFvFR, scFvADDLS, scFvFRS, scFv800DEK) and two tumor-targeting anti-HER2 immunogen conjugates (scFvFRS-E), scFv800DEK-E). For the computational procedures all the antibody structures were derived by homology modeling and assessed by molecular dynamics (MD) simulations. As regards the experimental section tobacco plants were transformed for stable and transient expressions. Specific expression vectors containing the gene encoding for the scFvS of interest were used. Transgenic plants were cultivated also in hydroponic and aero-copic systems. DNA, RNA and protein analyses were performed in leaves, roots and root exudations.

Results: Structural and MD analysis indicated a strong correspondence between structurally-determined flexibility of the binding site with the different functional behaviors proved by the wild-type and its mutant. Computational analysis of anti-HER2 immunogen conjugates showed that the presence of a toxin does not significantly affect the major physico-chemical parameters and their structure. The highest level of scFvS expression was observed in roots.

Conclusions: 1) The computational approaches represented a good tool for structural and functional characterization of the binding site. For analyzing physicochemical properties of immunogen conjugates and for predicting the effects of the linked toxin on structure, dynamics and functionality of the antibodies. 2) The proposed plant-based expression system seems to represent a promising tool for a large-scale scFv production.

ERHILL II – 2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich
Nürnberg, October 3-5, 2008

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Abstracts
Remifentanil: How it Relieves Human and Earthly Pain, and New Perspectives.

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Background: Remifentanil use has become a standard analgesic therapy during and after low-high complexity surgeries. Its indications have extended beyond surgery rooms and today, it is one of the drugs that has importantly changed the way of administering anesthesia for a considerable number of procedures specially for those that are ambulatory (out-patient surgery). However it is unknown what it’s impact will be on the emission of halogenated anesthetics which may be the origin of organic-halogenated pollutants (OHP) that are released into the atmosphere, resulting in ozone layer damage and more over these compounds are recognized as neurotoxic and endocrine modulators.

Conclusions: We determined the immediate cause of death of patients with penetrating cardiac injuries from the period July 1999 to July 2005. We determined the immediate cause of death of patients with penetrating cardiac injuries from the period July 1999 to July 2005. We determined the immediate cause of death of patients with penetrating cardiac injuries from the period July 1999 to July 2005.

Background: Interferon-y (IFN-y) is a pleiotropic cytokine with antiproliferative and immunomodulatory activities that are crucial for the regulation of immune responses. Methods: We examined a group of military pilots. The examinees were divided into 3 subgroups: ground personnel (9 persons, control group), 17 pilots with <1000 flight hours, and 12 pilots with >1000 flight hours. The quality of repair in many respects genetically determined; therefore, we used peripheral blood lymphocytes from pilots for in vitro detection of a radiodiphasic response (RAR), which was evaluated by the number of chromosomal aberrations.

Results: No differences in IFN-γ serum content after induction by NDV virus were detected. The adaptive response was observed in 7 individuals of the control group (78%), in 10 pilots who had <1000 flight hours (96%), and in 3 pilots having >1000 flight hours (33%). The examined individuals were divided into 2 groups depending on the presence of RAR, and IFN-y production after radiation was measured. It was shown that at doses 0.05 Gy or 0.5 Gy no differences between groups were detected. Exposure with these doses sequentially in 48 h interval resulted to differently directed changes: lymphocytes of individuals with RAR produced more IFN-γ than before while cells of persons without RAR made it less.

Conclusions: The quality of adaptive mechanisms evaluated by RAR may be useful for estimation of individual sensitivity to radiation during radiotherapy in oncology and in prediction of professional risk.

Differently Directed Changes in Interferon-γ Production Depending on Radioadaptive Response.

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Background: Interferon-y (IFN-y) is a pleiotropic cytokine with antiproliferative and immunomodulatory activities that are crucial for the regulation of immune responses.

Methods: We examined a group of military pilots. The examinees were divided into 3 subgroups: ground personnel (9 persons, control group), 17 pilots with <1000 flight hours, and 12 pilots with >1000 flight hours. The quality of repair in many respects genetically determined; therefore, we used peripheral blood lymphocytes from pilots for in vitro detection of a radiodiphasic response (RAR), which was evaluated by the number of chromosomal aberrations.

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Conclusions: The quality of adaptive mechanisms evaluated by RAR may be useful for estimation of individual sensitivity to radiation during radiotherapy in oncology and in prediction of professional risk.

Cardiac tamponade from slingshot metal darts in Chukh: a retrospective review of cases.

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Background: Polymorphisms of Cytochrome p450 2D6 (CYP2D6) have a significant effect on the pharmacokinetics of most antidepressants. Individuals are classified as poor metabolizers (PMs) due to inheritance of two mutant CYP2D6 alleles and develop higher plasma drug concentrations causing an increased risk of side effects and toxicity when subjected to standard recommended doses of CYP2D6 substrate drugs. In contrast individuals who are ultrarapid metabolizers (UMs) due to inheritance of alleles with duplicated or multuplicated active genes and will thus under circumstances not reach therapeutic plasma levels of CYP2D6 substrates, leading drug resistance and false accusation of non-compliance. Our study aimed to develop a rapid and reliable procedure used for early detection of PMs- associated mutations or deletions (CYP2D6 gene relative to the albumin gene as an internal standard gene using a quantitative PCR technique.

Results: One homozogous (16%), and three heterozygous (24% detectable alleles) and one patient had heterozygote gene duplication [4* gene duplication (2*1)]. Six individuals had gene duplication. Melting curves were verified using DNA samples of known genotypes and by sequencing the PCR products.

Conclusions: Our new genotyping procedure was evaluated against the ratio between the O-demethylated (ODV) metabolite of the CYP2D6 substrate Venlafaxine (V) and determined by HPLC serving as a quality control of the genotyped patients. This genotyping procedure was regarded as fast and reliable for clinical routine.
Manganese (Mn) Transport at the Blood-Brain Barrier: Implications for Parkinson’s-Like Disease

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Background: Though an essential trace element, exposure to high Mn levels has been implicated in a Parkinson’s-like disease, manganese. We hypothesized that symptoms associated with these two disorders reflect perturbations in shared molecular pathways. Accordingly, studies were carried out in C. elegans to test the hypothesis that Mn causes similar limitations because of synaptic toxicity, and (2) Mn preferentially targets dopaminergic (DAergic) neurons.

Methods: Bristol wild-type (WT) C. elegans N2 strain was used unless otherwise indicated. Over 10 years follow-up we conducted experiments to explain this phenomenon.

Conclusions: Amoebae in cysts were inoculated with OP50 and (2) Mn preferentially targets dopaminergic (DAergic) neurons. Direct injection of mebandazole and albendazole. J. Ultrasound. Med. 22, 469-478.

References:

Dexamethasone-Loaded anti-E-selectin Liposomes Prevents Glomerulonephritis Progression: The Potential of Vascular Bed-Specific Drug Delivery

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Background: Glomerulonephritis is a renal disease characterized by glomerular inflammation which is frequently treated with glucocorticoids. However, their use has been limited by systemic side effects and inefficacy. Therefore, we aimed to deliver dexamethasone-Mab to diseased kidney.

Methods: Glomerulonephritis was induced in C57Bl/6 mice and monitored for 2 weeks. Dexamethasone was injected intravenously. Gene expression was quantified in renal endothelial subsets after lacermicidosection. Disease parameters analyzed included the extent of glomerular crescent formation, proteinuria, glomerular hypertrophy and plasma glucose levels.

Results: E-selectin was expressed selectively by glomerular endothelial cells after induction of glomerulonephritis. Consequently, accumulation of anti-E-selectin (AbAsg) liposomes was 3.6 times higher than non-targeted IgG liposomes in diseased kidney. In glomerular dexamethasone-AbAsg liposomes co-localized with endothelial cells. Targeted delivery of dexamethasone-AbAsg liposomes reduced glomerular endothelial expression of P-selectin, E-selectin and VCAM-1 by 60% to 70%. Other renal microvasculature was not affected by targeted dexamethasone delivery and unlike administration of free dexamethasone, site selective delivery of dexamethasone-containing immunoliposomes and free dexamethasone were injected intravenously. Gene expression was quantified in renal endothelial subsets after lacermicidosection. Disease parameters analyzed included the extent of glomerular crescent formation, proteinuria, glomerular hypertrophy and plasma glucose levels.

Conclusions: Amoebae in cysts were inoculated with OP50 and (2) Mn preferentially targets dopaminergic (DAergic) neurons. Direct injection of mebandazole and albendazole. J. Ultrasound. Med. 22, 469-478.

References:
The changes in renal function after a single dose of intravenous furosamide in patients with compensated liver cirrhosis

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Background: Patients with compensated Child-A cirrhosis have sub clinical hypovolemia and diuretic treatment could result in renal impairment. Aim: To evaluate the changes in renal functional mass as reflected by DMSA uptake after a single injection of intravenous furosamide in patients with compensated liver cirrhosis.

Methods: Eighteen cirrhotic patients were divided in two groups; eight patients (group 1, age 56 ± 9.6 yrs, Gender M5/F3, 3 alcoholic and 5 non alcoholic) were given low intravenous 40 mg furosamide and ten other patients (group 2, age 54 ± 9.9 yrs, Gender M6/F4, 4 alcoholic and 6 non alcoholic) were given high 120 mg furosamide respectively. Renoscintigraphy with 100MBq Of Tc 99 DMSA was given intravenously before and 90 minutes after furosamide administration and SPECT Imaging was determined 3 hours later. All patients were kept under low sodium diet (80mEq/d) and all diuretics were withdrawn for 24 hours. Results: In patients with compensated cirrhosis the changes consist up to three hours after injection. This increase was as the same extent in either low or high doses of furosamide. (From 12.8% ± 3.8 to 15.2% ± 2.2, p 0.001 in Gr I as compared to 10.6% ± 4.6 to 13.5% ± 4.6 in Gr 2, p < 0.001). In 8 patients (45%, 3 pts from Gr 1 and 5 pts from Gr 2) DMSA uptake remain unchanged. The mean 8 hrs accumulation after intravenous furosamide was above 80 meq/l and was higher in Gr 2 as compared to Gr 1 respectively (136 ± 37 meq/l) VS 100 ± 36.6 meq/l, P 0.005). Finally, basal global renal DMSA uptake was decreased in 80% of patients; 22.5 ± 7.5% (Nl ± 40%), as compared to normal calculated creatinine clearance (CCT 101 ± 26), and measured CCT of 87 ± 30 cmin/m (P < 0.001).

Conclusion: A single furosamide injection increases renal functional mass as reflected by DMSA in 55% of patients with compensated cirrhosis and identify 45% of patients with reduced uptake and who could develop renal impairment under diuretics. Whether or not albumin infusion exerts beneficial effect in those patients with reduced DMSA uptake remains to be determined.

How to avoid drug—drug interactions

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Background: It may be favorable to use a combination of drugs, if the combination is well documented, to enhance the effect or to reduce adverse effects. However, in the case of patients visiting several different physicians, who are prescribing less appropriate combination of drugs due to being unaware of each other, the outcome may be negatively influenced. The polypharmacy may even result in serious adverse drug events.

Methods: In Sweden, the government has legislated and funded a nationwide mandatory database for all dispensed prescriptions. The information content is available during 15 months for prescribers and dispensing pharmacists, as well as for the registered individual. Prior to the launch of the nationwide database in Sweden, studies were performed to estimate the prevalence of potential drug—drug interactions (DDI) in a general population and to evaluate the historical change in risk over three decades.

Results: On average each individual filled 14.6 prescriptions during a 15 month study period (2003-2004). The risk of receiving a potential DDI was estimated as the cumulative incidence 0.26 overall. The relative risk for women was estimated as 1.3. For more severe potential DDIs the cumulative incidence was estimated as 0.02. The risk of receiving a potential DDI was positively correlated to age and polypharmacy. The change in risk over three decades increased for type C (relative risk RR 1.18), but decreased (RR 0.71) for the more severe type D interactions. Polypharmacy increased with more than 60% during the three decade study period. Fifteen months after launch of the new National Pharmacy Register in Sweden, the prevalence of individuals with dispensed drugs was 71% (6,424,457/9,047,752). For elderly (80-89 years) the mean number of dispensed prescriptions was 27.8 during the first 15 months.

Conclusions: The new National Pharmacy Register will provide health care professionals with a powerful tool to systematically review all prescriptions. Alert systems integrated in electronic healthcare records may be used to detect potential DDIs. To gain approval among physicians, the alerting should focus on the more severe and clinically relevant DDIs. More individual-oriented information (laboratory, genetic, allergies) may in the future be processed before prescribing of drugs, to better customize the therapy for the single individual.
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Background: Proliferating Schwann cells, the glial cells of the peripheral nervous system, are a prominent feature during early development and after damage to peripheral nerves. Altered Schwann cell proliferation is also associated with diseases and pathological states including inherited peripheral neuropathies, peripheral nerve tumors, and peripheral neuropathies secondary to diabetes, cancer chemo therapeutic agents, or toxins. To gain more insight into the molecular processes governing Schwann cell proliferation in health and disease, we examined the Schwann cell cycle and its regulation in vivo.

Methods: We have examined the expression, regulation, and localization of cyclins, cyclin-dependent kinases (cdk), and cell cycle inhibitors in Schwann cells of developing and adult peripheral nerves using immunohistochemistry. In addition, we used appropriate mutant mice to examine the functional requirement for the respective cell cycle proteins in Schwann cell proliferation.

Results: Proliferating Schwann cells during development express cyclin D1 in the cytoplasm. After injury, cyclin D1 becomes localized to the nucleoli of proliferating Schwann cells. Cyclin D1-deficient animals revealed that developmentally regulated proliferation is not affected by the absence of cyclin D1, whereas injury-induced proliferation is impaired. We further found that the cell cycle inhibitor p21 appears first in the cytoplasm of Schwann cells at postnatal day 7 when most cells have already ceased dividing. After nerve injury, however, p21 is localized mainly in nuclei of dedifferentiated Schwann cells. Consistently, p21-deficient Schwann cells did not detect and quantitate the apoptosis that accompanies nerve development and regeneration. In contrast, after nerve injury, nuclear p21 is required for correct cell cycle control at the peak of Schwann cell proliferation. We next investigated the requirements for cdk4, 2, and 6 during Schwann cell proliferation. We showed that only cdk2 and 4 are expressed in peripheral nerves. Our data from cdk-deficient mice indicate that postnatal Schwann cell proliferation is abolished in the absence of cdk4 but not in the absence of cdk2 or 6.

Conclusions: We find that distinct components of the cell cycle machinery that regulate Schwann cell proliferation during development differ fundamentally from those activated following nerve injury or in peripheral neuropathies.

Small Lipoprotein A-I subclasses (42,000-70,000) are Promising Biomarkers in Cardiovascular Disease

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Background: The predictive value of the high density lipoprotein (HDL) cholesterol as a biomarker for cardiovascular disease is now questionable. HDL contains several subclasses of varying size, composition, and function. Aims: 1) To detect and to quantitate the apolipoprotein A-I-containing lipoproteins (LpAI) in directly from fresh plasma instead of HDL subclasses. 2) To study the plasma distribution of these LpAI sub-classes in normolipidemics and hyperlipidemics. 3) To detect and quantify the apolipoprotein A-I-containing lipoproteins (LpAI) directly from fresh plasma instead of HDL subclasses. 4) To study the plasma distribution of these LpAI sub-classes in normolipidemics and hyperlipidemics. To follow up the changes in their distribution after a fatty meal and during pregnancy.

Method: Fresh plasma from 90 normolipidemics and 20 hyperlipidemics was examined. 12 different LpAI sub-classes of molecular mass ranging from 42,000 to >350,000 with Stokes radius of 2.96 to >5.8 nm. The percentage of the smallest subclasses (SlaA), (SlaB), and (SlaC), (50,000, 45,000, and 42,000) respectively was low in normolipidemics (7.8, 3.4, and 2.7%) and significantly (P < 0.01, 0.05, 0.05, respectively) higher in hyperlipidemics (15.9, 8.3, 5.2%, respectively). In normolipidemics, the level of SlaA increased significantly (P < 0.05) from the fasting level 4 h after fasting, and 4 and 6 h after a fatty meal. Two pregnant women were followed 4.5, 8.0, and 11.5 months during pregnancy and after delivery.

Results: We detected 12 different LpAI subclasses of molecular mass ranging from 42,000 to >350,000 with Stokes radius of 2.96 to >5.8 nm. The percentage of the smallest subclasses (SlaA), (SlaB), and (SlaC), (50,000, 45,000, and 42,000) respectively was low in normolipidemics (7.8, 3.4, and 2.7%) and significantly (P < 0.01, 0.05, 0.05, respectively) higher in hyperlipidemics (15.9, 8.3, 5.2%, respectively). In normolipidemics, the level of SlaA increased significantly (P < 0.05) from the fasting level 4 h after fasting, and 4 and 6 h after a fatty meal. Two pregnant women were followed 4.5, 8.0, and 11.5 months during pregnancy and after delivery.

Conclusions: 1) Significant differences in the distribution of SlaAI subclasses in plasma were detected in normo- and hyperlipidemics. 2) Their plasma level increases with the increase of plasma SlaAI. 3) They may be related to the lipoprotein A-I genetic lipoproteins. 4) The variation of SlaA level was more evident than the others and it may be a good candidate as a biomarker for cardiovascular disease.

Investigation of SGK-1 and D extras expression in Human Embryonic Kidney (HEK 293) cells

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Adrenal corticosteroids are involved in multiple aspects of CNS function — for example, the feedback inhibition of the hypophyseal-pituitary-adrenocortical axis. Typically, the action of glucocorticoids is mediated by rapid induction of mRNA and protein synthesis. Potential mediators of glucocorticoid action include dekarin1 (activator of GR protein signaling) 1) first reported as a rapidly induced mRNA in pituitary cells. The rapid induction of dekarin1 in response to glucocorticoids in pituitary cells raises the possibility that it may be involved in the sensitive in vitro glucocorticoid-mediated regulation of corticotropin secretion. In the present study we examined the induction of dekarin1 in human embryonic kidney (HEK293) cells which have been used as a model for glucocorticoid-mediated regulation of corticotropin secretion.

To verify that HEK 293 cells contain functional glucocorticoid receptors (GR) activated by demethylated, MMTV-LTR plasmid, which responds to activated GR by enhancing luciferase expression via the MMTV promoter, was transfected into the HEK 293 cells. 48 h after transfection, the cells were treated with demethylated. Analysis of the time-course of demethylated action on MMTV regulated luciferase activity revealed that luciferase induction was maximal at 120 min. Exposure to various concentrations of demethylated for 120 min produced an increase of MMTV regulated luciferase activity in HEK 293 cells in a concentration-dependent manner. Maximum effect was obtained with 100 nM demethylated. The expression of dekarin1 was assessed by Northern blot. The results of Northern blot showed a 5 kb dekarin1 and a 2.6 kb band in glucocorticoid-induced protein kinase (SGK-1) mRNA (a positive control for glucocorticoid induction) species in HEK 293 cells. Demethylated has no significant effect to amount of dekarin1 mRNA induction for varying times (0-120 min) but increased SGK-1 mRNA with maximum effect at 30 min. The analysis of SGK-1 protein in HEK 293 cells also showed significant increase in response to dekarin1.

The results suggest that; 1) HEK 293 cells respond to demethylated via an endogenously expressing GR; 2) Dekarin1 is not a glucocorticoid-induced protein in HEK 293 cells; 3) SGK-1 is induced by demethylated in HEK 293 cells.

All abstracts are listed in alphabetical order of the presenting author.
Targeted delivery of cisplatin using polymeric nanoparticles

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Background: Cisplatin use is associated with serious side effects. The more selective delivery (targeting) of cisplatin to tumor cells would reduce drug toxicity, improving its therapeutic index. Aim: To develop long-circulating cisplatin-loaded nanoparticles and test their anticancer activity in mouse cancer models.

Methods: A) Nanoparticles preparation: Cisplatin-loaded poly(lactide-co-glycolide)-polyethylene glycol (PLGA-PEG) nanoparticles were prepared by a double emulsion method. B) Determination of cisplatin concentrations in blood circulation: BALB/c mice were administered intravenously with PLGA-PEG/cisplatin nanoparticles and at predetermined time intervals blood samples were obtained and assayed for cisplatin. C) Evaluation of the tolerance of BALB/c mice to the PLGA-PEG/cisplatin nanoparticles: 3 groups of mice received PLGA-PEG/cisplatin nanoparticles with a cisplatin content of 2.5 or 10 mg cisplatin/Kg. The fourth group of mice received blank nanoparticles and the fifth group of mice (n=3) received 100 μl saline. D) Evaluation of anticancer activity of PLGA-PEG/cisplatin nanoparticles: HT 29 tumor cells were injected sub-cutaneously into the left flank of SCID mice. Fifteen days later, the mice (n=6-8) were injected intravenously 5 times at weekly intervals with free cisplatin or cisplatin-loaded nanoparticles at the same dose (5 mg/Kg on a cisplatin basis).

Results: The entrapment of cisplatin in the nanoparticles resulted in a significant prolongation of cisplatin presence in blood. Balb/c mice tolerated 3 weekly intravenous injections of a relatively high dose of blank PLGA-PEG nanoparticles (500 mg/Kg) and 3 weekly intravenous injections of a high dose of nanoparticle-entrapped cisplatin (10 mg/Kg). The cisplatin-loaded PLGA-PEG nanoparticles was effective at delaying tumor growth in HT 29 tumor-bearing SCID mice. The group of mice treated with cisplatin-loaded nanoparticles exhibited higher survival rate compared to the free cisplatin group.

Conclusions: 1) PLGA-PEG/cisplatin nanoparticles could prolong cisplatin residence in blood and they were well tolerated by normal Balb/c mice even when relatively high doses were administered to mice. 2) The PLGA-PEG/cisplatin nanoparticles reduced tumor growth in SCID mice with HT29 xenografts, and these mice exhibited higher survival rate than free cisplatin.

The Effect of Melatonin and Zinc on the Immune Response in Experimental Toxoplasma Retinochoroiditis

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Background: Toxoplasmosis is a zoonotic disease and the most common cause of infection of the human retina in humans and animals, the primary control mechanism being to target the vasculature of specific organs, different tumors, or selected sites of infection. One of the potential beneficial approaches to improve the immune defense against T. gondii infection is zinc supplementation, since due to its catalytic and regulatory functions; zinc can enhance resistance to infections. Other potential approaches that deserve further investigation in this respect is the effect of melatonin (MEL) deficiency and artificial MEL supplementation on the immune response to T. gondii infection. MEL is a hormone secreted by pineal gland that has both direct and indirect effects on the immune system. In the current study, we investigated the impact of MEL and/or zinc deficiencies and artificial MEL and/or zinc supplementations on immune and inflammatory responses in the rat model of toxoplasma retinochoroiditis.

Methods: Eighty-four Sprague Dawley male rats were divided into 12 equal groups. All groups, except controls were infected with T. gondii parasite by intraperitoneal injection. Combinations of zinc deficient diet, pimeleocin (P), artificial zinc, and MEL were supplied during a 1 month period. At the end of the experiment, retinal and choroidal total lymphocytes, CD3+, CD4+, and CD8+ cell numbers were counted in histological sections.

Results: The highest amount of cellular infiltration (lymphocytes, CD3+, CD4+, CD8+ cells) in the choroid and retina were detected in infected=MEL+zinc treated rats and the least amount of cellular infiltration was observed in P+zinc deficient diet treated rats. Although single zinc or MEL supplementation had no significant impact on the cellular infiltration in the retina and choroid in P rats, combined therapy significantly improved these responses.

Conclusions: Artificial supplementation of MEL and zinc should be considered as an adjunctive therapy to classic treatment of Toxoplasma retinochoroiditis especially in immunosuppressed and elderly patients if our data will be confirmed in a clinical setting.

All abstracts are listed in alphabetical order of the presenting author.
Insulin, IGF-1 and Rosiglitazone: How Do They Effect The Glucose

Abstracts  Page A-21

Preventing Immune Evasion as a Strategy for Enhancing the Effectiveness of Herpes Simplex Virus Vaccine.

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Background: Herpes simplex virus-1 (HSV-1) and HSV-2 glycoprotein C (gC) are immune evasion proteins that inhibit complement activation by binding complement component C3b. We previously reported that mice passively immunized with a gC1 monoclonal antibody (mAb) were protected from HSV-1 challenge only if the mAb blocked the interaction between gC1 and C3b. We also demonstrated that immunizing mice with gC1 protein induced antibodies that blocked C3b binding and protected mice against HSV-1 challenge by blocking immune evasion.

Methods: We use the mouse flank (epidermal disease) model to evaluate whether immunizing with gC1 protein increases the efficacy of a glycoprotein D (gD1) subunit vaccine when challenged with HSV-1. We first defined a gD1 immunizing dose that produced partial protection against HSV-1 challenge. Our rationale for partial protection was that the gD2 subunit vaccine used in human trials provided only limited protection, and we wanted to reproduce these results in mice. We then immunized mice with either gD alone or with both gD and gC and challenged with HSV-1.

Results: We found that when gC1 was added to gD1 immunizations, mice were significantly protected from epidermal disease compared with gD alone. Importantly, the combined immunizations were more effective than gD alone in preventing infection of dorsal root ganglia. Passive immunization of anti-gC1 IgG obtained from mice immunized with gC1 protein protected complement intact mice, but not C3K knockout mice.

Conclusion: We conclude that immunizing with gC1 blocks immune evasion and enhances the efficacy of a gD1 subunit vaccine.

Levetracetam in the Treatment of Neuropathic Pain: Evidence from Cellular and Behavioral Pain Models

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Background: Neuropathic pain is a common, complex, costly pain condition, "initiated or caused by a primary lesion or dysfunction in the nervous system", presenting a major healthcare and social problem. Despite an increasing number of trials there is currently no satisfactorily effective therapy for neuropathic pain. Similarities between the pathophysiological phenomena observed in some epilepsy models and in neuropathic pain models justify the use of anticonvulsants in the symptomatic management of neuropathic pain. We attempted to elucidate the effectiveness of levetraacetam in neuropathic pain state in mice model and subsequently cellular mechanisms involved in a cellular nociceptive model.

Methods: The in vivo nociceptive behavioral "hot-plate test" was performed in normal and diabetes (streptozocin 200 mg/kg i.p.) induced adult male BalbC mice.

Subsequent to behavioral testing, electrophysiological measurements and calcium imaging experiments were performed on cultured neurones of the rat dorsal root ganglia (DRG) using the whole cell patch-clamp technique and the fura-2 ratiometric fluorescence microscopy. Data were analyzed using Kruskal-Wallis One-way Analysis of Variance (ANOVA), Dunnett's and Students' t tests, where appropriate.

Results: While levetraacetam had (60-900 mg/kg) no significant effect on the nociceptive threshold in normal mouse much lower doses (≤200 mg/kg) significantly restored the threshold in diabetic mice, in a dose-dependent manner. Current clamp recordings from DRG cells indicated that levetraacetam caused membrane hyperpolarisation and reduction of multiple action potential firing. Estimation of reversal potential and the hyperpolarizing currents indicated involvement of K+ channels. Furthermore, levetraacetam dose-dependently suppressed the depolarisation (high K+)- induced intracellular calcium signals in DRG neurons.

Conclusions: Results obtained from in vivo behavioral tests and cellular electrophysiological and ratiometric fluorescence measurements in rat sensory neurones with agreement lend support to the validation of the promising therapeutic potential of the new anticonvulsant levetraacetam for the management of neuropathic pain.

Antimicrobial Resistance by Mycoplasma species in Farm Animals.

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Background: More than 125 Mycoplasma species have been identified, many being pathogens in farmed animals. Mycoplasmas lack a cell wall, hence they are not susceptible to penicillins and some other antimicrobials. Mycoplasma infections are usually treated with tetracyclines, macrolides, chloramphenicol, and fluoroquinolones, however antimicrobial resistance to all of these has been reported. The treatment of mycoplasma infections is rarely successful and often requires repeated treatments. Although Mycoplasma species are not usually zoonotic, this repeated use of antimicrobials and development of resistance may have implications for the critically important antimicrobials used in human health. MIC data from UK bovine and ovine mycoplasma isolates and resistance mechanisms in M. bovis is presented.

Methods: In vitro microbroth dilution minimum inhibition concentrations (MIC's) were determined for 14 antimicrobials against M. bovis and M. ovipneumoniae isolates. Sensitive M. bovis isolates were subcultured at sub-MIC levels in 14 different antimicrobials to induce antimicrobial resistance in vitro. Whole cell antimicrobial resistance had been induced this variant DNA was used to PCR the domains II and V of the 23S rRNA gene and the gyrA and parC genes, which were then sequenced.

Results: Isolates of M. bovis and M. ovipneumoniae gave high in vitro MIC values against most classes of antimicrobials, except the fluoroquinolones, although high fluoroquinolone MIC’s have since been recorded in field isolates. The rate of development of antimicrobial resistance varied between isolates and between antimicrobials, but resistance was induced by all isolates to all antimicrobials. Fluoroquinolone resistant isolates showed a single base change (G to A) at position 248 in the gyrA gene, observations that have been shown to confer fluoroquinolone resistance in E. coli.

Conclusions: It is unlikely that the generic exchange of resistance genes occurs between Mycoplasma species and zoonotic organisms, however the treatment of Mycoplasma infections in farmed animals requires careful selection and monitoring of antimicrobial use to maximise the effectiveness of treatment and to evade the further development of antimicrobial resistance.
EM703 as a New Derivative of Erythromycin, Inhibits Lung Fibrosis Induced by TGF-β1 Signaling in Murine and Human Lung Fibroblasts

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Background: 14-membered ring macrolides have been effective in chronic airway inflammation and also prevented lung injury and fibrosis in bleomycin-challenged mice via anti-inflammatory effects. EM703 is a new compound of Erythromycin (EM) without bactericidal effects. We investigated the anti-inflammatory and antifibrotic effects of EM703 in 1) experimental murine fibrosis model induced by bleomycin and 2) murine and human lung fibroblast cell lines.

Methods: 1) Seven weeks old male ICR mice (eight mice/group) were used. Bleomycin was administered intravenously to mice at day 0. EM703 was orally administered daily to mice. All groups were examined for cell populations in bronchoalveolar lavage fluid, and for induction of mRNAs of Smad3 and Smad4 in lung tissues by RT-PCR at day 7. Fibroblastic foci were assessed histologically and hydroxyproline content was chemically determined in lung tissues at day 28. 2) We also assessed proliferation and soluble collagen production, and examined induction of mRNA of Smad3 and Smad4 by RT-PCR in lung fibroblast cell line Mlg2908. We examined smad3, smad4, smad7 and phosphorylated smad2/3 (P-smad2/3) protein assay by western blotting in lung fibroblast cell lines.

Results: Bleomycin-induced lung fibrosis, infiltration of macrophages and neutrophils into the alveolar space were inhibited by EM703. Expression of smad3 and smad4 mRNA was significantly attenuated by bleomycin, but recovered by EM703. EM703 also inhibited fibroblast proliferation and the collagen production in lung fibroblasts induced by TGF-β1. Expression of smad3 and smad4 mRNA in murine lung fibroblasts disappeared by TGF-β1, but recovered by EM703. EM703 inhibited expression of phosphorylated-smad2/3 and smad4 protein in murine lung fibroblasts induced by TGF-β1.

In human lung fibroblast, EM703 inhibited the augmentation of Smad3 mRNA induced by TGF-β1. Inhibited Smad3 mRNA by TGF-β1 was augmented by co-incubation with EM703.

Conclusions: These findings suggest that EM703 improves bleomycin-induced pulmonary fibrosis in mice by actions of anti-inflammation and regulation of TGF-β1 signaling, which is associated with inhibition of phosphorylation of Smad2/3 through recovery of Smad7 level.

Effects of Alcohol and Sucrose Intake on Rat Liver Cyp2e1

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Background: Ethanol metabolism by CYP2E1 leads to a significant reactive oxygen species (ROS) release, accompanied by the defense systems decrease against oxidative stress. Since expression of CYP2E1 is very much influenced by nutritional factors, specially carbohydrate consumption, and various results concerning the expression of CYP2E1 were obtained with low-carbohydrate alcohol liquid diet or the intragastric tube feeding model that also utilizes a low carbohydrate diet, this study describes the effects of ethanol and sucrose treatment on CYP2E1 levels using an ad lib model.

Methods: Male Sprague-Dawley rats were fed ad. lib. for 1, 2, 3 or 4 weeks a commercial diet (Purina Ind., Brazil) plus a 25% ethanol-20% sucrose solution. Control groups were isocalorically pair-fed to the leading ethanol-consuming animals, or received isocaloric amounts of sucrose for pairing only ethanol calories. Eighteen hours before sacrifice ethanol was withdrawn and animals had only access to tap water or they were offered food and water ad lib.

Results: Our results have shown that ethanol administration was associated with CYP2E1 induction, otherwise CYP2E1 stabilization was more associated to sucrose consumption.

Conclusions: Our findings indicate that dietary deficiencies, especially low carbohydrate intake could be crucial in the CYP2E1 stabilization.
Significant Interactions between some Antibiotics and Antimalarial Drugs

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**Background:** Coadministration of antibacterial and antimalarials is common in the tropics as a result of frequent association of malaria with other infections. The combination of ampicillin and cloxicillin (AM-CL) prescribed in many infections produces a broad-spectrum antibiotic activity against both Gram-positive and Gram-negative bacteria. The current studies investigated effects of chloroquine(CQ), proguanil(PG), quinine(QH) and artesunate(ART) on the bioavailability and activity of cloxicillin( Clox) and ampicillin(Amp) in vivo and in vitro.

**Methods:** 7 healthy volunteers received single oral doses of Clox alone followed by Clox + PG, another 8 volunteers received AM-CL alone and AM-CL+ CQ, while another 14 volunteers received AM-CL alone and AM-CL + ART in a cross-over manner. Total urine voided was collected at various time intervals. Clox in urine was determined by HPLC. Effect of CQ and PG on dissolution of Amp and Clox and on their antibacterial activity against E. coli and S. aureus was investigated.

**Results:** CQ showed a significant decrease in total amount (Du=%) and % dose of Clox excreted in urine by 64%, while PG led to 48% decrease. Ongoing study reveals 90% reduction of Clox by CQ. In vitro dissolution revealed >40% reduction in % Amp and Clox dissolved in the presence of CQ. Similar results were obtained with PG. The MIC of Amp was increased two- to four-fold from 5.42 µg/ml to 10.83 & 21.66 µg/ml by CQ, an indication of reduction in bactericidal activity of Amp. Similar results were obtained between Clox and CQ. 9 out of 14 subjects (64%) showed 39% decrease in urinary excretion of Clox while only 5 (36%) showed an increase of 27% when AM-CL were coadministered with ART.

**Conclusion:** These results indicate significant drug-drug interactions between AM-CL and antimalarials in a way that correlates in vitro with in vivo findings. The urinary pharmacokinetic studies indicate marked reduction in bioavailability of Clox when coadministered with CQ, PG and QN and ART. The in vivo interactions may be due to interference with the dissolution of Amp and Clox in the body by the antimalarials. Though clinical implications of the findings are inconclusive, it should be careful in prescribing these classes of drugs together to avoid sub-therapeutic levels, which can lead to treatment failure and drug resistance.

**Transdermal Drug Delivery into and Beneath the Skin - Application to Anti-Inflammatory Drugs**

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**Background:** α-Melanocyte-stimulating hormone (α-MSH) is an endogenous neuro-immunomodulatory peptide that has potent anti-inflammatory effects. We hypothesize that this compound can be developed as a topical formulation for the therapy of psoriasis and contact dermatitis. The stability of α-MSH, and in vitro and in vivo percutaneous absorption from various dermatological vehicles was investigated in rats. The anti-inflammatory effect of topical delivery of α-MSH was tested on an allergic contact dermatitis (ACD) mouse model.

**Methods:** The stability of α-MSH in ethanol-water (1:9) at various temperatures (40 to 70°C) and pH conditions (pH 1.0 to 10.0) was examined. In vitro permeation and skin retention kinetics of α-MSH as a saturated solution in various dermatological vehicles were studied using hairless rat skin and Franz diffusion cells. The in vivo skin penetration of α-MSH was studied in rats by a dermal microdialysis technique. The anti-inflammatory effect of selected topical formulations (0.25 and 0.5% of α-MSH in 10% n-methyl-2-pyrrolidone and 50% ethanol) was evaluated in the ACD mouse model.

**Results:** Stability studies indicated that α-MSH possesses an ~110 day shelf-life (time of 10% degradation) as determined by HPLC. Further, α-MSH demonstrated good stability in the pH region between 3.0 and 8.0. Permeation studies indicated that ethanol, transcutol and propylene glycol (PG) and ethanol vehicles had maximum permeation of α-MSH through the skin (between 5.0 and 7.5 µg / 24 h). Ethanol demonstrated the maximum skin retention (2.0 µg/mg) as compared to all other vehicles for which the skin retention was < 1.0 µg/mg. Dermal microdialysis results show the ethanol formulation produced a maximum concentration (Cmax) in dermis of 6.46±2.21 ng/ml as compared with the PG formulation with a Cmax of 2.56±1.284 ng/ml, demonstrating 2.5 fold higher dermal levels by the ethanol formulation. α-MSH formulations demonstrated significant anti-inflammatory activity in an ACD mouse model. The data indicate that like desametasone, α-MSH was effective in reducing the ACD response.

**Conclusions:** Stable α-MSH can be formulated for effective topical delivery into skin layers to demonstrate significant anti-inflammatory activity in an ACD mouse model.
Background: Diverse insults increase the amount of unfolded/misfolded proteins in the endoplasmic reticulum (ER). This leads to activation of a defensive unfolded protein response, which is controlled by ER-resident protein, GRP78/BiP. Whether the cell dies or survives the ER-stress is determined by the amount of available GRP78/BiP. Recent discovery that GRP78/BiP is the only known ERtarget for the proteolytic A subunit (SubA) of a novel bacterial Abi toxin provides the first opportunity for targeted destruction of GRP78/BiP. Aims: To establish if targeted delivery of SubA into tumor cells is selectively cytotoxic. 2) To establish if the DNA binding capacity of such compounds was retained. Methods: SubA was genetically fused to human epidermal growth factor (EGF) and the resulting EGF-SubA was expressed in E. coli. Tumor cells (MCF7, PC3, F98, F98-EGFR, MDA231Luc) were treated with EGF-SubA alone or in combination with various drugs. Biochemical and cell biology methods were used to characterize cellular EGF levels, GRP78/BiP cleavage, activation of unfolded protein response, apoptosis, and cell viability. Results: Exposure of cells expressing high levels of EGF to EGF-SubA resulted in rapid (~2 hours) EGF-mediated destruction of GRP78/BiP. Despite the ongoing cleavage, in most cells it results in significant upregulation of GRP78/BiP level by 24h of treatment. EGF-SubA is highly cytotoxic to growing and confluent cells with high level of EGF (> 10^7 EGF per cell), per cell with EGF in the range of 3 to 40 pM, while EGF-nerve cells are at least 500-fold less sensitive. EGF-SubA strongly synergizes with staurosporine, an inhibitor of proapoptotic drug, with additive potency of the drug compound alone. Less prominent synergism is observed with drugs that are less stressful for ER. Conclusions: 1) In vitro, pan-EGF receptor antagonist SubA might be a new “magic target”. Its targeted destruction with subnanomolar concentrations of EGF-SubA is extremely cytotoxic, while non-toxic concentrations of EGF-SubA disarm cellular defense and allow to use virtually non-toxic amounts of ER-stress inducing drugs.

Antineoplaston: Synthesis, Biological, and Clinical Evaluation in Egypt

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Background: Antineoplastons, first described by Burzensky, are naturally occurring peptides and amino acid derivatives, which control neoplastic growth. Antineoplaston A-10 (3-phenylacetylamino-2,6-diaminopropionic acid) is the prototype A10. We previously reported the utility of antineoplaston A-10 to inhibit tumor growth in multiple experimental models. Here we evaluated antineoplaston A-10 against A10.

Methods: Antineoplaston A-10 level was measured in the urine of 31 breast cancer patients and 17 normal women using high performance liquid chromatography (HPLC). We determined the optimal concentration of A-10 in vitro on apoptosis was tested in vitro after adding A-10 at a concentration of 10 ng/mL to the cellular suspensions of breast cancer patients. Only those cases without previous treatment for breast cancer were included. Neutrophil apoptosis was evaluated in patients with breast cancer at time of diagnosis and to correlate urinary antineoplaston A-10 levels with neutrophil apoptosis and to describe the relationship to the presenting author.

Results: Correlate urinary antineoplaston A-10 levels with neutrophil apoptosis and to describe the relationship to the presenting author.

Conclusions: Antineoplaston A-10 may provide rational basis for designing trials to employ DNA binding capacity in breast cancer patients. These findings confirm the presence of immune defects among patients with breast cancer and thus the need to develop new strategies to induce and augment immunity for the treatment of breast cancer.

The Strong Growth Advantage in Stationary Phase Phenomenon in Mixed Cultures of Antimicrobial Resistant Escherichia and Salmonella

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Background: During prolonged stationary phase mutants with increased fitness express growth advantage in stationary phase (GASP) enabling them to grow and displace the parent as the dominant population. Aims: 1) to study the GASP phenotype in mixed cultures of Escherichia coli and Salmonella enterica serovar typhimurium wild type and strain harboring either the resistance to nalidixic acid (Nal) or to streptomycin (Str). 2) To evaluate the influence of multiresistance on the appearance of GASP phenotype. 3) To detect if there are epigenetic dynamic differences between both aged wild-type and used resistant mutants (Str and Nal) of E. coli, the pulsed-field gel electrophoresis (PFGE) of total genomic DNA was conducted.

Methods: In a typical GASP competition experiment, cells from a 10-day-old culture are inoculated as a numerical minority (1:100 vol/vol) into a young (1-day-old) culture. The genomic DNA from randomly chosen colonies was digested with SfiI and resolved by pulsed-field gel electrophoresis (PFGE).

Results: In the mixture consisting of the aged S. enterica Str and young E. coli Nal, strong phenotype GASP mutants of S. enterica dominated after the third day of mutual growth. Likewise, but with inverse bacterial resistance, in the mixture of 10-day-old S. enterica Nal culture with young culture of E. coli Str, S. enterica Nal GASP mutants of strong phenotype dominated the mixture after the fifth day and were maintained at about 1×10^4 CFU/ml. Electroretrophoretic karyotype of 10-days-old GASP mutants of E. coli Nal revealed additional bands when compared to the wild-type.

Conclusions: 1) The strong phenotype GASP is obtained in mixed cultures with the aged mutant strains, but not when the isogenic antibiotic-sensitive strains were used. 2) The cells in mixed cultures of double mutant E. coli strain Nal Vol5 bare significantly higher antiproliferative agents than A10 and tamoxifen. They also had significantly higher activity on a human breast cancer cell line against the prototype A10 and the antibreast drug tamoxifen. Moreover, the DNA binding capacity of such compounds was retained.

Antibacterial, Antisecretory and Antihemorrhagic Activity of Azadirachta indica Used to Treat Cholera and Diarrhea in India

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Background: Indigenous uses of Azadirachta indica A. juss (Malacae) (locally known as neem) leaves in different parts of India for curing gastrointestinal disorder such as diarrhea and cholera is wide spread. The objective of the present study was to evaluate the antibacterial and antisecretory activity of neem leaf extract against Vibrio cholera, a causative agent of watery diarrhea such as cholera.

Methods: Methanolic extract of neem leaves was tested using the strains of multi-drug resistant V. cholerae belonged to O1, O139 and non-01 serotypes. Antibacterial activity of the extract (10, 25, 50, 100, and 200 mg/ml) was determined by agar-diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were assessed using the broth microdilution method. The effect of the extract on fluid secretion and hemorrhage in intestine induced by V. cholerae was studied using mouse model (Male BalB/C mice, 23.8 ± 1.1 g body weight). Mice of each of groups 1 and 2 (n=2 per group in duplicate) were administrated 500 µl of bacterial inoculum (2×10^6 CFU/ml) orally. After 1 h mice were fed either with 200 µl of methanol (group 1) or crude extract (group 2); at oral doses between 100 and 1800 mg/Kg in NaCl (0.9%) solution (1 ml/mouse) orally. Mice were sacrificed after 24 h incubation and fluid accumulation (FA) ratio was measured.

Results: Crude extract showed inhibitory activity against multi serogroup strains of V. cholerae by agar-diffusion assay with significance (p<0.00). MICs reached by 50% (MIC1) and 90% (MIC2), and MBC for the extract were 2.5 mg/ml, 50mg/ml and 10 mg/ml respectively. Addition of the extract (100 mg/ml) did not produce any sign of toxicity in mice. Group 1 mice showed fluid accumulation (FA ratio; between 0.11±0.01 and 0.16±0.02) and hemorrhage in the intestines. Neem extract showed activity with inhibition of (0.9%) of fluid secretory values of 27.7 ± 7.6, 41.1 ± 3.4, 43.3 ± 1.3, 57.0 ± 5.9, and 77.9 ± 7.2 % at doses of 100, 200, 300, 450, and 900 mg/kg respectively. Oral administration of the extract inhibited hemorrhage induced by V. cholerae in mouse intestine at a dose ≥300mg/kg (visually observed).

Conclusions: 1) Methanolic extract of A. indica leaves was an effective antibacterial agent against V. cholerae, and significantly reduced the fluid secretion and hemorrhage induced by V. cholerae in mouse intestine. 2) The active extract may be employed for the treatment of cholera and as potential source to develop new antimicrobial compound and antibacterial drug useful to treat cholera and diarrheal patients.

Authors' disclosure statement: This work has been published in J. Ethnopharmacology. Thakurta P, Bhowkim S, Mukherjee T. K, Hajra A, Patra and P. K. Bag 2007. Antibacterial, antisecretory and antihemorrhagic activity of Azadirachta indica used to treat cholera and diarrhea in India. J. Ethnopharmacology (Elsevier) 111 (3): 607-611.

All abstracts are listed alphabetically in the present author's naming.
The Frequency-Dependent Effect of Infrasound on Bull Sperm Velocity

BAGULEY BC. Antivascular therapy of cancer: DMXAA. may not only provide a basis for improved clinical therapy but also facilitate it itself, secondly by hypoxia and thirdly by cytotoxic drugs. Studies with DMXAA antitumour activity of tumour macrophages may be increased firstly by DMXAA macrophages through toll-like receptors such as TLR4. Thus, the potential antitumour activity of tumour macrophages may be increased firstly by DMXAA uptake and intracellular level of cGMP and cGMP.

Methods: Experiments were performed on preliminary frozen bull sperm incubated in 2.5% NaClate water solution at 30°C. 16 treatment of sperm samples was done by means of IS waves generating by a special setup. Ca-uptake and intracellular cyclic nucleotides contents (cAMP and cGMP) were measured by Wallac 1405 liquid scintillation counter. The sperm velocity was recorded by means of digital video camera (SONY, Japan) set on biological microscope MB-14B connected to PC and sperm direct velocity was estimated by Pinnacle studio program.

Results: It was shown that 2Hz, 30dB IS 5min treatment caused the pronounced elevation effect (25%, ***: p<0.001) on the sperm velocity (Figure 1), which was accompanied by decreasing of intracellular level of c-GMP by 26±0.7% (*: p=0.05), and increasing of intracellular level of c-AMP 43.5±1% (‡: p<0.005) and Ca-uptake 285±6% (‡*: p<0.05) by sperm.

The action of a new drug that targets a cancer’s blood supply: the story of DMXAA (ASA404)

BAGUELY BC

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DMXAA (ASA404; 5,6-dimethylxanthene-4-acetic acid), developed in this laboratory [1,2], is currently undergoing Phase III clinical trial, in combination with the cytotoxic drugs carboplatin and paclitaxel, in patients with non small cell lung cancer. DMXAA was originally developed as the most active member of a series of analogues of the drug flavone acetic acid [3]. DMXAA has both a direct, early effect on tumour blood vessels, leading to inhibition of tumour blood flow, and a later effect that is mediated by local release of cytokines and other vasoactive molecules. It is this double action that maintains a sufficiently long duration of tumour blood flow arrest to induce irreversible damage and vascular collapse [1]. The effect DMXAA on tumour vascular endothelial cells appears to be mediated by p38 MAP kinase but the signalling pathway in tumour macrophages that leads to increased production of cytokines has not yet been identified. DMXAA facilitates a positive feedback loop where cytokines from tumour macrophages reduce blood flow, leading to inhibition of tumour blood flow, and a later effect that is mediated by local release of cytokines and other vasoactive molecules. It is this double action that maintains a sufficiently long duration of tumour blood flow arrest to induce irreversible damage and vascular collapse [1]. The effect DMXAA on tumour vascular endothelial cells appears to be mediated by p38 MAP kinase but the signalling pathway in tumour macrophages that leads to increased production of cytokines has not yet been identified. DMXAA facilitates a positive feedback loop where cytokines from tumour macrophages reduce blood flow, leading to inhibition of tumour blood flow, and a later effect that is mediated by local release of cytokines and other vasoactive molecules. 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Are Grapefruit, Orange, Lime, Pummelo and Apple the Forbidden Fruits of Drug Interactions?

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Background: Intestinal drug metabolism and carrier-mediated drug transport are accredited as fundamental factors affecting systemic drug availability. This presentation will initially review inactivation of CYP3A4 by grapefruit, Seville orange, lime and pummelo juices and in isolated enterocytes. OA TP1A2 was the only shown uptake transporter of the DNA synthesis and an excellent target of cancer chemotherapy. Resveratrol, a naturally occurring stilbene derivative, is a potent free radical scavenger exerting a multitude of biochemical and antiinflammatory effects. Resveratrol was identified as an inhibitor of ribonucleotide reductase (RR), a key enzyme of DNA synthesis and an excellent target of cancer chemotherapy. Inhibitors of RR have been previously shown to exert synergistic combination effects with cytarabine, a well-established antileukemic agent. We investigated the biochemical effects of resveratrol and its polyhydroxylated derivative, 3,3',4,4',5,5'-hexahydroxystilbene (M8) on the in situ RR activity, cell cycle distribution, induction of apoptosis and inhibition of NF-kappaB. Furthermore, it was tested whether a combination of resveratrol or M8 with cytarabine could yield synergistic cytotoxic and apoptotic effects in human HL-60 promyelocytic leukemia cells.

Methods: Cytotoxic effects of resveratrol, M8 and cytarabine alone and in combination were analyzed using growth inhibition and clonogenic assays. Synergistic combination effects were identified by the CalcuSyn software. In situ inhibition of RR was determined by the incorporation of [3H]-labelled cytidine into the DNA of resveratrol-treated HL-60 cells. The IC50 values of resveratrol and M8 was measured by a HPLC method. Induction of apoptosis was studied using a Hoechst/propidium iodide staining method. Induction of TNF-alpha induced activation of NF-kappaB was shown by EIA and Western blotting and cell cycle distribution was analyzed by FACS.

Results: Resveratrol effectively inhibited incorporation of [3H]-labelled cytidine into DNA. Furthermore, incubation of HL-60 cells with resveratrol significantly decreased intracellular dCTP, dTTP, dATP and dGTP concentrations. M8 depleted intracellular NTP pools and dTTP as well as dATP pools. Moreover, M8 inhibited the activation of NF-kappaB and arrested HL-60 cells in the G2-phase of the cell cycle. Based on these results, we investigated the combination effects of resveratrol and M8 with cytarabine. In growth inhibition, apoptosis and clonogenic assays, resveratrol and M8 acted synergistically with cytarabine in HL-60 cells.

Conclusions: Based on the observed in vitro-in vitro synergistic effects, the combination of cytarabine with resveratrol or M8 could become a viable option in the chemotherapy of leukemia and therefore deserves further testing.
Cytokine-associated neutrophil extracellular traps and antineural antibodies in Plasmodium falciparum infected children under the age of six

Methods: Peripheral blood smear slides and blood samples obtained from 21 Nigerian children under six years of age, presenting with uncomplicated malaria before and seven days after initiation of sulfadoxine-pyrimethamine (SP) treatment were analyzed. The slides were stained with Giemsa and with DAPI. Levels of the pro-inflammatory cytokines IFN-γ, IL-1, IL-6, CRP, and IL-4, selected anti-inflammatory cytokines TGF-β and IL-10, and ANA were determined by immunofluorescence.

Results: The children exhibited circulating NETs with adherent parasites and erythrocytes, elevated ANA levels, a Th2 dominated cytokine profile, and IL-4, IFN-γ, IL-10, and ANA were determined by immunofluorescence.

Conclusions: The results of this investigation suggest that NET formation and ANA to dsDNA may induce pathology in falciparum-infected children, but activate a protective mechanism against falciparum malaria in adults. The significance of in vivo circulating chromatin in NETs and ssDNA ANA as a causative factor in the hyporesponsiveness of CpG oligonucleotide-based malaria vaccines is discussed.

Search for diagnostic and prognostic markers for Glioblastoma multiforme (GBM).

Methods: Peripheral blood samples were obtained from 31 patients with GBM and 15 healthy controls. The following techniques were used to analyze glioblastoma specimens:

- a) Microarray analysis
- b) SELDI-TOF mass spectrometry
- c) Laser optical tweezers
- d) DIGGE studies
- e) Real-time PCR
- f) ELISA

Results: Several novel proteins and genes were identified that differentially express in Glioblastoma specimens in comparison to normal control brain samples.

Conclusions: There are several genes that are either downregulated, lost of function or upregulated that we observed in our research. These are working further on the importance of these genes and their proteins in their role as a significant biomarker by analyzing more patient population.

Synthesis and Biological Evaluation of Anticancer β-Lactams

Methods: The following techniques were used to synthesize β-lactam analogues: a) Microwave synthesis, b) Laser optical tweezers of DIGGE and e) Real-time PCR.

Results: Several novel proteins and genes were identified that differentially express in Glioblastoma specimens in comparison to normal control brain samples.

Conclusions: There are several genes that are either downregulated, lost of function or upregulated that we observed in our research. We are working further on the importance of these genes and their proteins in their role as a significant biomarker by analyzing more patient population.

A Novel Anti-Angiogenic Glycotherapeutic for Breast Cancer

Methods: The following techniques were used to synthesize β-lactam analogues: a) Microwave synthesis, b) Laser optical tweezers of DIGGE and e) Real-time PCR.

Results: Several novel proteins and genes were identified that differentially express in Glioblastoma specimens in comparison to normal control brain samples.

Conclusions: There are several genes that are either downregulated, lost of function or upregulated that we observed in our research. We are working further on the importance of these genes and their proteins in their role as a significant biomarker by analyzing more patient population.
Synthesis of a Series of 8-Substituted-Phenylxanthines and a Study on the Effects of Substitution Pattern of Phenyl Substituents on Affinity for Adenosine A1 and A2A Receptors: The Magic of Theophylline

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Background: Adenosine receptors are promising therapeutic targets in a wide range of conditions, including cerebral and cardiac ischaemic diseases, sleep disorders, immune and inflammatory disorders and cancer. 8-Phenyltheophylline is the parent member of a variety of potent adenosine receptor antagonists. Herein we report the synthesis of a new series of 8-substituted-phenylxanthines and the effects of substitution pattern of phenyl substituents on adenosine receptors binding affinity.

Methods: The three series of compounds A, B and C were prepared by treating 5,6-diamino-1,3-dimethyluracil with alkylaminalkyl substituted derivatives of vanillin, isovanillin and 3-hydroxybenzaldehyde and subsequent cyclization in thionyl chloride.

Results: The compounds were evaluated for their affinity for A1 and A2A receptors using [3H]DPCPX and [3H]ZM 241385 as radioligands. Table summarizes the observed affinities of various newly synthesized 8-phenylxanthine derivatives in radioligand binding assays at human A1 and A2A receptors. The presence of a methoxy substituent at 3 or 4- position of phenyl ring in A and B along with an ortho polar side chain increases selectivity for A1 over A2A receptors. However, absence of a methoxy group as in C results in almost equal selectivity for both subtypes.

Table: Adenosine A1 and A2A binding affinities of compounds

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>A1 (µM)</th>
<th>A2A (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>B</td>
<td>2.1</td>
<td>0.095</td>
</tr>
<tr>
<td>C</td>
<td>0.54</td>
<td>0.064</td>
</tr>
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Conclusions: It can be concluded that suitable selection and positioning of aryl substituents may lead to development of potent and selective xanthine based adenosine receptor antagonists.

The Modified Vaccination Technique developed by Barabas provides the "magic bullet" for the prevention and cure of chronic ailments such as autoimmune disorders and cancer specifically, without side effects

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Background: Barabas has developed and implemented a new vaccination method called modified vaccination technique (MVT) - in experimental animals - that can be employed both prophylactically and therapeutically with equal effectiveness to combat chronic disorders such as autoimmune diseases, cancer and exogenous antigen induced mishaps. This breakthrough discovery of antigen presentation by the MVT allows the induction of a predetermined beneficial immune response outcome.

Presently available vaccination programs are preventative (through active immunization) or therapeutic (through passive immunization) and neither technique can evoke specific downregulatory (in autoimmune disorders) or upregulatory (in cancer and chronic infections) immune responses to correct chronic disorders.

Methods: When vaccinating, using the MVT, appropriately assembled immune complexes - made up of the specific target antigen and specific homologous polyvalent or monovalent antibodies against the target antigen - have to be prepared (the "magic bullets") for the prevention and treatment of presently drug only treatable conditions such as autoimmune diseases and cancer. The goal is to inject exactly the right immune complex (the "magic bullet") into recipients to evoke the production of the same class of immunoglobulin (i.e., antibody) with the same specificity against the target antigen that resides in the inoculum.

Results: Using the MVT and antibody information transfer is achieved resulting in predetermined immune responses. Through the utilization of the MVT the potential of downregulating or upregulating immune events - in humans with certain autoimmune disorders and cancer - to achieve self cure and regained tolerance to self is in sight.

Conclusion: We believe absolutely in the immune system's natural ability to correct mistakes. It requires only in certain instances tailored made "instructions" for eliciting and maintaining redirected beneficial immune-response outcomes.

Aldosterone modulation of hormone receptors in cancer treatment

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Background: Triolostane was originally developed and used as an inhibitor of 3-beta hydroxysteroid dehydrogenase in treating Cushings' syndrome. It was later found to confer clinical benefit in breast cancer patients. Our studies began with the premise that triolostane (at micromolar concentrations) could effect the function of the estrogen receptor (ER) but without displacing the native ligand, estradiol, from its receptor binding site. We have proposed that triolostane can act as an allosteric modulator of the ER and our research has focused on trying to find an explanation for its efficacy in the treatment of postmenopausal breast cancer.

Methods: In these studies we have used a wide range of biochemical and functional methods, including ligand-binding assays, gene reporter assays, gene expression microarrays and cell proliferation assays, in order to try to define an alternative mechanism of action for triolostane in the context of breast cancer.

Results: Triolostane does not displace radio-labelled estradiol from estrogen receptor preparations. It inhibits MCF-7 breast cancer cell proliferation at concentrations of 1µM and above. It has direct inhibitory effects on ER function and events mediated through oestrogen response elements (EREs) and activating protein-1 motifs. In a study of the effects of triolostane and tamoxifen, on MCF-7 cells using microarrays, striking differences were found in gene expression profiles. Interestingly, triolostane was found to selectively up-regulate the expression of the beta subtype of estrogen receptor (ERβ), an effect replicated in vivo. This is significant as tamoxifen-resistant breast cancer is often associated with reduced ERβ protein expression and triolostane can inhibit proliferation of tamoxifen-resistant MCF-7 cells in vitro.

Conclusions: Our data support previous clinical findings that triolostane can influence the effectiveness of tamoxifen and may subvert tamoxifen resistance, and demonstrates in a wider context that allosteric modulation of receptor function may be a useful therapeutic approach in circumstances in which the receptor ligand binding domain is occupied by its cognate ligand or an antagonist. This concept has potential application in clinical situations in which either nuclear receptors or surface membrane located receptors are implicated in cancer progression.
Erythromycin: Magic Bullet for Sore Throat and Its Consequences in Children

BASNET NB1, BASNET SB1

1Children’s Medical Diagnosis Center (CMDC), Kathmandu, Nepal; 2Durga Bhawan Polyclinic, Kathmandu, Nepal.

Background: Most people suffer from sore throat particularly in the first two decades of life. Previous studies have proved that sore throat is a major cause of sickness and a potentially dangerous source of infection in children. We evaluated the clinical situation of sore throat in Nepalese children, and reviewed literature to understand mechanism erythromycin as its magic remedy.

Methods: We prospectively evaluated the clinical data of children below 19 years who visited Children’s Medical Diagnosis Center and Durga Bhawan Polyclinic from January 2006 to December 2007. All patients were consulted and the final diagnosis was made by a consultant pediatrician and pediatric cardiologist. We also analyzed all the publications indexed in PubMed up to June 27, 2008 on key words children and sore throat and erythromycin conducted in various studies.

Results: Of the total 1175 examined patients 159 (13.53%) had tonsillitis. Amongst 159 patients 63.53% were between 4 to 10 years. The male female sex ratio was 1.48. Our clinical experience supplemented by reported 150 literature studies have showed that erythromycin has been used in all age groups and sex, is effectively acceptable, well tolerated orally, and also available in parenteral form. It has been available worldwide after its discovery by McGuire and coworkers in 1952 from a strain of Streptomyces erythraeus, originally obtained from soil in the Philippines. It is able to eradicate a broad range of bacteria including streptococci, staphylococci, listeria, legionella, diphtheria, pertussis, tetanus, syphils and Campylobacter jejuni without any fatal toxic effect. It exerts effective concentration on tonsilar tissues. Erythromycin has both curative (e.g. sore throat) and prophylactic (e.g. rheumatic fever, rheumatic heart disease, acute glomerulonephritis) benefits in man. It has been proved effective in treating sore throat and other infections thereby decreasing its untoward life threatening cardiac, renal, respiratory and neurological complications, as well as the cost of treating cardiac consequence, such as valvular surgeries.

Conclusions: Erythromycin has reliable chemotherapeutic effect in treating sore throat and preventing its cardiac, respiratory, renal and neuronal consequences in children of developing and industrialized nations thus proving its role as a 'magic bullet'.

IRMS. Lasers have recently been employed to measure the analysis, analytical methodology has relied upon expensive and cumbersome portable and affordable device. Clinical applications. The laser spectrometer has a great potential to be a success story.

Results: E rapidly increased ROS production in cancer cells. Sustained oxidative stress led to activation of the intrinsic mitochondrial apoptosis pathway, with cardioprotin oxidation, decreased mitochondrial membrane potential, cytochrome c release and caspase activation. Antioxidants blocked all activities of E. ROS increase and apoptosis preferentially occurred in cancer cells relative to normal cells. E inhibited tumor growth as a single-agent and synergized with P in causing tumor regression in human xenograft tumor models. At 21 US sites, 53 subjects were randomized to E+P and 28 subjects to P. The addition of E to P yielded a doubling survival rate. The median PFS (112 weeks) relative to normal cells, and are more susceptible to further ROS increases which push the cell beyond its oxidative stress tolerability limit. In this way, E takes advantage of a fundamental hallmark of cancer to selectively kill cancer cells with little effect on normal cells. In a randomized, blinded Phase 2 clinical trial, E+P resulted in a clinically meaningful, statistically significant increase in PFS, with an acceptable toxicity profile and encouraging survival data. A global Phase 3 trial (SYM-METRY(1)) of E+P in chemotherapy-naïve stage IV metastatic melanoma patients is ongoing.

Methods: The in vitro activity of E was assessed in human Hs295T melanoma, Ramos lymphoma and HSB2 leukemia cell lines. Preclinical efficacy was tested in an M14 melanoma human xenograft model in mice. Stage IV metastatic melanoma patients were randomly assigned to 213 mg/m(2) E plus 80 mg/m(2) P (E+P) or 80 mg/m(2) P as a weekly one-hour intravenous infusion, three of every four weeks until disease progression or RECIST or death. The primary efficacy endpoint was progression-free survival (PFS). Introduction: The blood-brain barrier (BBB) possesses a highly concentrated transporter, the P-glycoprotein (P-gp) transporter. It protects the brain from several endogenous and exogenous compounds by acting as an active cell membrane efflux pump. Changes in P-gp function have been implied in several neurological conditions. Uproregulation of P-gp has been found to prevent uptake of drugs in brain tumours and in epilepsy, leading to therapy-resistance. Decreased P-gp function has been associated with Amyloid-β transport and progression of Alzheimer’s disease (AD). Also, it has been related to toxin exposure and the development of Parkinson’s disease (PD). Finally, age-associated decline in P-gp function may facilitate the accumulation of toxic substances in the brain. We studied in vivo BBB P-gp function in older healthy subjects and in patients with PD.

Methods: BBB P-gp function was studied using positron emission tomography (PET) and (11C)-verapamil. 17 healthy volunteers with age 18-68, 22 patients with Parkinson’s disease in different disease phases and six subjects with parkinsonism (PSP and MSA) were scanned. Distribution volume (Dv) of the tracer in the brain was calculated using Logan analysis. Statistical Parametric Mapping (SPM2) was used to study specific regional differences between the subject groups.

Results: Older subjects showed significantly increased brain tracer uptake as compared to the younger healthy subjects, indicating decreased BBB P-gp function. In PD, the more advanced patients showed increased tracer uptake in frontal regions, while de novo patients showed decreased uptake. In patients with Parkinson’s, increased uptake was detected in the brain stem, substantia nigra, and basal ganglia regions.

Conclusion: Decreased blood-brain barrier P-gp function with aging could be a mechanism by which age acts as the main risk factor for the development of neurodegenerative disease. It also means that several drugs will reach higher concentrations in the brains of elderly patients. The knowledge that P-gp function can be modulated by drugs could open the way to new therapies for neurodegenerative disease.

Elesclomol: A Novel Oxidative Stress Inducer for the Treatment of Metastatic Melanoma

BARSOUM J, FOLEY KP, WILLIAMS A, JACOBSON E, ODAY S, 4783-03

Background: Elesclomol (E) is a small molecule drug candidate that stimulates production of reactive oxygen species (ROS) and selectively induces cancer cell apoptosis. The clinical efficacy of E in combination with paclitaxel (P) was investigated in a double-blind, randomized, controlled Phase 2 trial in melanoma.

Methods: The in vitro activity of E was assessed in human Hs295T melanoma, Ramos lymphoma and HSB2 leukemia cell lines. Preclinical efficacy was tested in an M14 melanoma human xenograft model in mice. Stage IV metastatic melanoma patients were randomly assigned to 213 mg/m(2) E plus 80 mg/m(2) P (E+P) or 80 mg/m(2) P as a weekly one-hour intravenous infusion, three of every four weeks until disease progression or RECIST or death. The primary efficacy endpoint was progression-free survival (PFS).

Results: E rapidly increased ROS production in cancer cells. Sustained oxidative stress led to activation of the intrinsic mitochondrial apoptosis pathway, with cardioprotin oxidation, decreased mitochondrial membrane potential, cytochrome c release and caspase activation. Antioxidants blocked all activities of E. ROS increase and apoptosis preferentially occurred in cancer cells relative to normal cells. E inhibited tumor growth as a single-agent and synergized with P in causing tumor regression in human xenograft tumor models. At 21 US sites, 53 subjects were randomized to E+P and 28 subjects to P. The addition of E to P yielded a doubling survival rate. The median PFS (112 weeks) relative to normal cells, and are more susceptible to further ROS increases which push the cell beyond its oxidative stress tolerability limit. In this way, E takes advantage of a fundamental hallmark of cancer to selectively kill cancer cells with little effect on normal cells. In a randomized, blinded Phase 2 clinical trial, E+P resulted in a clinically meaningful, statistically significant increase in PFS, with an acceptable toxicity profile and encouraging survival data. A global Phase 3 trial (SYM-METRY(1)) of E+P in chemotherapy-naïve stage IV metastatic melanoma patients is ongoing.

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Application of Safron and Its Ingredients as a Pharmaceutical Herb from Ancient Times and the Mechanisms of Their Action

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Background: Safron (Crocus sativus L.), which is named as 'red gold' in Iran, has been used not only as a food additive and a dye, but also as a medicinal herb in Iran from ancient times. In the last two decades, it has been reconsidered because of its various biological properties. Chemical analysis has shown the presence of more than 150 components in saffron stigmas. In this presentation we reviewed the historical and medicinal uses of saffron in different parts of the world, especially in Iran where saffron was cultivated for the first time (in addition to the wild type), and where nowadays is the biggest saffron producer (more than 80% of world saffron cultivation). Then, the new findings from our laboratory as well as other research groups, about its medicinal properties and various cellular and molecular mechanisms of action were discussed.

Methods: Web of Science and Medline were searched for saffron and its constituents. For ancient uses of saffron, manual searching of Persian books and searching internet resources, among with manual searching of their references were done. In our experiments, after purification of important constituents from Iranian saffron, their application on breast and gastric cancer were studied in model animals; their molecular mechanism of action was also investigated using in vitro experiments.

Results: Saffron was known from more than 3000 years ago by Iranians, Assyrians Babyloniens and Minos; not only for its application as a food additive, dye and perfume, but also for its usage as a medicinal herb (alone or in combination with other drugs) to treat a wide range of diseases. Newly, its application as an important medicinal herb and other cellular and molecular mechanisms of its action are also under study. Saffron's more powerful components were carotenoids and monoterpenes. Structure-function relationship studies show that some properties are related to deglycosylated derivatives, while others belong to more glycosylated ones.

Conclusion(s): Saffron as an important medicinal herb is a good candidate to be considered for new drug design.
Inhaled H₂S for suspended animation in anesthetized and ventilated mice

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1Klinik für Anästhesiologie und 2Unfallklinik, Universitätsschilm, Ulm, Germany

Background: Several organs can reversibly slow down their vital functions in order to sustain otherwise lethal environmental stress, a phenomenon called hibernation. In mice, inhaling hydrogen sulfide (H₂S) induced such a hibernation-like state called “suspended animation” with decreased heart rate, respiratory minute volume and metabolic expenditure, and consecutively hypothermia. H₂S also is an endogenous gaseous messenger and signaling molecule, and both pro- and antiinflammatory effects were reported. Since in rodents anesthesia per se causes hypothermia, we tested the hypothesis whether inhaling H₂S may also induce suspended animation in anesthetized, mechanically ventilated and instrumented mice.

Methods: 15 hours after laparotomy animals received 100 ppm H₂S or vehicle for 5 hours with core body temperature maintained at 38° or 27°C. Left ventricular pressure-volume loops were assessed using a pressure-conductance catheter. Cytokine (TNFα, IL-6) and chemokine (MCP-1, MIP-2) formation, myeloperoxidase and NF-κB activation were measured in tissue biopsies, mitochondrial respiration was measured in liver biopsies (high-resolution respirometry).

Results: In contrast to awake mice, H₂S alone did not affect hemodynamics. Within 3 hours, however, both hypothermia alone and combined with inhaled H₂S decreased heart rate, respiratory minute volume and metabolic expenditure, while stroke volume, ejection fraction and end-diastolic pressure were unchanged. While inhaled H₂S and hypothermia alone comparably attenuated lung chemokine levels, only hypothermia caused attenuation of tissue IL-6 levels. Strikingly, H₂S significantly increased NF-κB activation during normothermia. Combining hypothermia and H₂S in anesthetized H₂S-treated mice induced -cyclo-oxygenase–stimulated mitochondrial respiration.

Conclusions: 1) In contrast to awake mice anesthesia blunted the hemodynamic effects of inhaled H₂S alone. 2) H₂S has anti-inflammatory properties beyond hypothermia alone. 3) Reduced cyclo-oxygenase–stimulated inhibition of mitochondrial respiration suggests attenuated mitochondrial damage. References: 1. Blackstone et al. Science 2005;308:518

Acknowledgement: Supported by the Deutsche Sepsis-Gesellschaft

Gastrointestinal Prokinetic 5-HT₄ Agonists; Receptor Selectivity and Benefit-to-Risk Profile

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Background: Selective 5-HT₄ receptor agonists (e.g. TD-5108 and prucalopride) appear to produce more robust gastrointestinal (GI) prokinetic activity in patients with chronic constipation compared to both non-selective agonists (e.g. tegaserod or cisapride) and placebo. Interaction with non-5-HT₄ receptors may also result in adverse effects; cisapride at 5-HT₄ receptor affinity (mean pKᵢ = 7.5, 8.4, 7.0, 7.2 and 7.2 for TD-8954, tegaserod and cisapride, respectively; TD-5108 and TD-8954 had the highest potency, in dogs, po administration; while atracurium, volume, ejection fraction and end-diastolic pressure were unchanged. While inhaled H₂S and hypothermia alone comparably attenuated lung chemokine levels, only hypothermia caused attenuation of tissue IL-6 levels. Strikingly, H₂S significantly increased NF-κB activation during normothermia. Combining hypothermia and H₂S in anesthetized H₂S-treated mice induced -cyclo-oxygenase–stimulated mitochondrial respiration.

Conclusions: 1) In contrast to awake mice anesthesia blunted the hemodynamic effects of inhaled H₂S alone. 2) H₂S has anti-inflammatory properties beyond hypothermia alone. 3) Reduced cyclo-oxygenase–stimulated inhibition of mitochondrial respiration suggests attenuated mitochondrial damage. References: 1. Blackstone et al. Science 2005;308:518

Acknowledgement: Supported by the Deutsche Sepsis-Gesellschaft

Gut Health-Promoting Adhesion Of Enteropathogens To Dietary Polysaccharides

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Background: Bacterial adherence to host tissues is regarded as an important initial step for colonisation and infection. Hence, agents that interfere with the ability of pathogens to adhere to gut cells are promising antioxidants. Different plant products and food stabilizers were tested in terms of their binding capacity for enteropathogenic bacteria using a miniaturised adhesion test.

Methods: Bacterial strains were allowed to adhere to fibrous substances (Table 1) in triplicate in two independent assays each. Means were compared by Fisher’s unprotested limited least significant difference test. Results: Table 1 shows the performance of various substances in terms of their binding capacity for different E. coli and Salmonella enterica. Substances with the shortest detection time bound most bacterial cells (Becker and Galletti, 2008).

Table 1. Detection times of growth (h) of different E. coli and Salmonella enterica. Substances with the shortest detection time bound most bacterial cells (Becker and Galletti, 2008).

<table>
<thead>
<tr>
<th>Substances</th>
<th>E. coli</th>
<th>Salmonella enterica</th>
<th>Detection time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm kernel meal</td>
<td>9.73</td>
<td>9.36</td>
<td>2.64</td>
</tr>
<tr>
<td>Locust bean gum</td>
<td>9.52</td>
<td>9.40</td>
<td>3.37</td>
</tr>
<tr>
<td>Konjac gum</td>
<td>9.35</td>
<td>9.40</td>
<td>3.59</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>9.23</td>
<td>9.40</td>
<td>3.75</td>
</tr>
<tr>
<td>L-carnitine</td>
<td>9.17</td>
<td>9.40</td>
<td>3.91</td>
</tr>
<tr>
<td>Coenzyme A</td>
<td>9.17</td>
<td>9.40</td>
<td>3.96</td>
</tr>
<tr>
<td>Folic acid</td>
<td>9.23</td>
<td>9.40</td>
<td>4.02</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>9.23</td>
<td>9.40</td>
<td>4.15</td>
</tr>
<tr>
<td>Niacin</td>
<td>9.23</td>
<td>9.40</td>
<td>4.24</td>
</tr>
<tr>
<td>Thiamine</td>
<td>9.23</td>
<td>9.40</td>
<td>4.37</td>
</tr>
<tr>
<td>Biotin</td>
<td>9.23</td>
<td>9.40</td>
<td>4.49</td>
</tr>
</tbody>
</table>

Conclusion: With growth as measured variable for adhesion, a simple high-throughput method was developed and applied for the screening of large numbers of different food and feed components and bacteria. References: Becker PM et al. (2007). J. Appl. Microbiol. 103, 2686-2696.


Authors' acknowledgements: This study was funded by the Dutch Product Board of Animal Feed (PVD) and by SAFEWASTES (EU project no. 513949) in equal shares.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts Page A-30
Background and aim: Many extended-spectrum β-lactamases (ESBL) producing isolates of E. coli and K. pneumoniae are susceptible in vitro to amoxicillin-clavulanic acid (AMC), ceftazidime-clavulanic acid (CAZ/ci), and pipercillin-tazobactam (TZP) but MICs increase substantially when higher inoculum is applied. The aim of this study was to determine the effect of inoculum size on the susceptibility of E. coli and K. pneumoniae isolates with well characterized ESBLs to amoxicillin (AMX), AMC, cefazidime (CAZ), CAZ/ci, pipercillin (PIP), TZP, imipenem (IMI) and meropenem (MEM).

Material and methods: Minimum inhibitory concentrations (MICs) were determined by broth microdilution method using inocula that differed 100 fold in density according to CLSI. The inocula contained 10^5 CFU/ml and 10^3 CFU/ml approximately. The study was performed on the set of K. pneumoniae and E. coli strains producing SHV-2, SHV-5, SHV-12, CTX-M-3 and CTX-M-15 β-lactamases.

Results: Inoculum effect for CAZ/ci was detected in 52% of SHV-2 producing K. pneumoniae strains followed by AMC (43%) and TZP (38%). SHV-6 producing K. pneumoniae strains showed the most pronounced inoculum effect with CAZ/ci (57%) and AMC (55%) and to lesser extent with TZP (44%). Inoculum effect was observed with all strains of K. pneumoniae and E. coli producing SHV-5 β-lactamase showed the most pronounced inoculum effect with AMC (61%) followed by CAZ/ci (61%) and TZP (55%). Strains producing CTX-M β-lactamases had a marked inoculum effect with CAZ/ci (71%), AMC (57%) and TZP (50%). AMC and CAZ/ci were associated with inoculum effect against all type β-lactamases. CAZ/ci, AMC and TZP were the most affected by the inoculum size then AMC, and CAZ/ci particulary with CTX-M producers. The activity of TZP was mostly compromised in the presence of high density of SHV-5-producing K. pneumoniae.

Conclusions: Carbapenems were the most stable compounds to inoculum effect regardless of the type of ESBL.
The Path from Colles’ Law to the “Magic Bullet”

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The hypothesis that syphils might be counteracted immunologically can be traced to the Irish surgeon, Abraham Colles, who noted in 1837 that a congenitally syphilitic infant may be born of a mother who showed no signs of syphils. He interpreted this to indicate that the infant, infected by a diseased mother during fertilization, immunizes itself against syphilis, even though it can infect other caretakers. This talk will sketch the disappointing investigations that led from “Colles’ law” to the reception of Ehrlich’s “magic bullet.”

The first line of serologic investigation, initiated by Joseph Azuz-Turenne in the 1840s was called “syphilization.” In this controversial therapy the patient was “saturated” over several months with hundreds of subcutaneous injections of “syphilitic toxins.” Based on the hypothesis that when saturation was reached new manifestations of the disease were prevented, after which cure might be achieved. Saturation had been achieved when injections no longer elicited a local inflammatory reaction. Syphils and chancre had not been differentiated at this time. Thus the misinterpretation of “saturation” of syphilitic patients was frequently compounded by the injection of chancroidal rather than syphilitic serum. Most responsible for perpetuating this technique until about 1870 was the Norwegian venereologist, Caesar Boeck.

With the advent of bacteriology it became of interest why certain species were resistant to a pathogen that was lethal to another. Could this “resistance factor” be transferred, either as prophylaxis or therapy? The first injections of serum from animals that could not be made syphilitic were undertaken in 1890 on patients with secondary syphilis and were recognized to be ineffective. Further trials were stimulated by favorable reports by an Italian venereologist who in 1892 gave subcutaneous injections of lambs’ blood. Several investigators used serum from dogs, rabbits, sheep, and horses. When the use of serum from untreated animals became recognized as useless, donor animals were pre-treated with syphilitic “toxin” or mercury. The greatest stimulus to experimentation with serum therapy came in 1893 with the announcement of successful serum treatment of syphilis and yaws. The lack of a proven pathogen of syphils added confusion to the search for an anti-syphilitic serum.

The immediate acceptance of the discovery in 1895 of the Spirchopta pallida as the pathogen, rapidly followed by discovery of a practical diagnostic method, may explain why, in the midst of the belief that a curative serum for all microbial diseases was about to be discovered, Ehrlich’s announcement in 1910 of the therapeutic efficacy of medicinal “Salvarsan” was received so enthusiastically. Ehrlich was more circumspect regarding the usefulness of Salvarsan than many of its early advocates.

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Abstract: Rickettsiae grow only intracellularly. The antibiotic susceptibility is assessed by plaque, dye uptake or IF assays. Rickettsiae are susceptible to doxycycline, thiamphencol and fluoroquinolones. Bclalactams, aminoglycosides and cotrimoxazole are not active. Typhus group rickettsiae are susceptible to all macrolides, whereas the spotted fever are more resistant to rifampicin than the other rickettsiae. Rickettsiae feis is not susceptible to gentamicin, erythromycine, amoxiciline or cotrimoxazole. We present an overview of susceptibility of rickettsiae to antimicrobials.

BENDTSEN K

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Background: Today, recombinant gene technology permits the use of drugs which are almost identical with natural human proteins, including antibodies (Abs). Many assume that these drugs pose little or no risk of triggering specific immune responses, because patients according to dogma are tolerant towards their own proteins. Unfortunately, this is not the case, and even so-called 100% human biologicals may be immunogenic. I shall focus on the immunogenicity of anti-TNF Abs constructs plus the largely ignored problem of individual variations in drug bioavailability (BA) and pharmacokinetics (PK).

Methods: Several methods have been used to assess circulating levels of anti-TNF biologicals as well as Abs to these drugs. Most are based on solid-phase technology, e.g. ELISA, with their inherent problems of low sensitivity, false positivity and susceptibility to npspecific interference, fx by rheumatoid factors. We have developed fluid-phase radioimmunoassays (RIAs) for monitoring patients on anti-TNF biologicals, one for functional blood levels of the drugs, and one for anti-drug Abs.

Results: We measured BA/PK and anti-drug Abs developed in “anti-TNF immunized” patients with rheumatoid arthritis; most of these were treated with infliximab. The most sensitive anti-drug Ab assay involved binding to soluble and intact infliximab rather than to plastic-immobilized drug. Indeed, data obtained by solid-phase assay using cross-binding of plastic-fixed and soluble infliximab were inconsistent with results obtained with fluid-phase RIA. Despite intravenous administration, there were sizable interindividual variations in serum trough levels of the drugs even at time-points where anti-drug Abs had not yet developed; these levels diminished or disappeared in parallel with Ab induction (30% and 44% of patients on infliximab were Ab-positive at 3 and 6 months, respectively). Abs were ‘neutralizing’ in that their levels were positively associated with inhibition of TNF binding to the drugs. There were highly significant correlations between high levels of anti-drug Abs and later dose increases, side-effects and cessation of therapy.

Conclusions: To prevent prolonged use of inadequate anti-TNF biotherapies, individualized assessments of BA/PK and Abs seem essential (personalized medicine). In our hands, fluid-phase assays are superior to solid-phase assays.
A Study of Cepacetinibe and Cisplatin in the Treatment of Recurrent Carcinoma of the Uterine Cervix

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Background: Platinum is the mainstream of treatment in advanced or recurrent cervical carcinoma; however, the duration of response is short lived as well as the median survival. Fluorouracil (5-FU) has been shown to be active in cervical carcinoma. Cepacetinibe, an oral fluoropyrimidine carbamate, is sequentially converted to 5-FU by systmine phosphorhase (TPF) which is found at higher concentrations in cervical carcinoma than normal tissue. In addition, cisplatin further upregulates TP. Cepacetinibe plus cisplatin has the potential to be an active treatment, which is more convenient than 5-FU-cisplatin.

Methods: This study combines cepacetinibe and platinum in patients with recurrent cervical carcinoma with no potentially curative standard treatments. Sixteen patients (14 squamous cell carcinoma, and 2 adenocarcinoma) with a median disease-free interval of 11 months (range, 2-86) received cepacetinibe 50 mg/m² intravenously on day 1 and oral cepacetinibe 1000 mg/m² twice daily for two weeks with a one week rest period.

Results: Median age was 50 years (range, 31-74). A total of 89 cycles were administered with a mean of 5.5 cycles (range, 3-6) per patient. Four of the sixteen patients had complete response (25%), 4 had partial response (25%), and 5 had disease stabilization (31%). Ten patients (63%) had recurrent disease outside the radiation field. The overall response rate in patients with recurrent disease within the previous irradiated field was 33% and 60% in patients with tumor outside the irradiated field. The median follow-up time was 29 months (range, 11-39). The median time to progression was 9 months (range, 5-37), with a median overall survival of 23 months (range, 5-37). The majority of adverse events were mild and there were no grade 4 adverse events. Hematological toxicity was the most frequent adverse event with grade 3 neutropenia in 19% of patients. Grade 2 and 3 hand-foot syndrome occurred in 38% and 6% of patients, respectively. There were no chemotherapy-related deaths.

Conclusions: This active yet convenient combination of cepacetinibe and cisplatin shows a high response rate, long time to progression and survival with acceptable toxicities for patients with recurrent carcinoma of uterine cervix.

Giroline, a new antiplasmodial lead extracted from the sponge Cymbastela cantharella

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Background: Malaria is the most prevalent parasitic disease in the world today. In this context, there is clear demand to search for new antimalarial agents and research into new antimalarial drug candidates originating from natural sources has been actively pursued. Giroline (Figure 1), a 2-aminoimidazole derivative extracted from Cymbastela cantharella (a New-Caledonian Sponge) already known for its antitumor activity, was tested against Plasmodium.

Methods: We evaluated the effects of giroline and some of its analogues in vitro and in vivo against P. falciparum and P. vivax strains, respectively. We then evaluated its toxicity in vitro and in vivo. We have also determined the point of action of giroline in the erythrocytic life cycle of the malarial parasite and its synergic action with chloroquine.

Results: We have demonstrated that giroline presents a very promising activity against malaria both in vitro against four P. falciparum strains and in vivo on murine model. Giroline also showed a specific mode of action by inhibiting Plasmodium protein synthesis. Moreover, between giroline and chloroquine, a clear synergic effect was noted.

Conclusions: Giroline is of a real interest as research basis for a new class of antimalarials. With such a biological profile, giroline could be considered as a model chemical structure for new candidates in the arsenal of new drugs in particular of drugs able to fight malaria.

Natural Products (NP) - Microbial Metabolites (MM) - Antibiotics (AB): History, Facts and Problems, Where Now?

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History: Great benefit and unpredictable negative consequences. The NP research and especially the discovery of new MM declined in the past years. Few new drugs were discovered. The reasons are: resistance-problem, less success with HTS and combinatorial chemistry, but the reasons are mainly economic and regulatory. In the last years the total synthetic efforts and the discovery of new compounds from higher plants and marine organisms increasing, but sometimes no evidence of the activity of the produced species. (endophytes, taxoids, patellamide).

Facts: Close to half a million NP, including ~100000 MM, 20000 microbial AB (~350 marketed), there are known. High percentage, (~40 %) of all drugs and about 70-80 % of all known AB drugs derived from NP, as direct drugs, derivatives (semisynthetic and modified products), and other NP mimics (synthesised as NP analogue). The NP libraries have some advantages over random synthetic or combinatorial chemical libraries in several respects (e.g. complexity, high biological activity, complex structures hardy accessible by chemical methods). "Nature is the best combinatorial chemist". They also meet the greener chemistry. The bioprodutos have inherent - but perhaps undiscovered - biological functions and drug-like structures, compatible to the host. The real function of MM is the communication with other microbes, higher organisms and the environment. They are the interface of the viruses in order to be prepared for yet unknown risks for the future life span.


Possible Consequences of Transplacental Transfer of Viruses in Healthy Pregnants (Review)

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Division of Virology, National Center for Epidemiology, Gyáli Str. 2-6, H-1097 Budapest, Hungary

Background: Viruses, which may cause illnesses of the fetus were shown to be transferred frequently through the placenta into fetal tissues without any clinical consequences. Rubella and rubella vaccine, measles and measles vaccine, Toxoviruses, Flaviviruses, hepatitis A virus (HCA), Hepatitis B virus (HBV), human cytomegalovirus (HCMV), human herpesvirus types 1 (HSV), 6 (HHV6), 7 (HHV7), and 8 (HHV8), human parvovirus B19 (HPV-B19), dermatovirus (AAV), human adenosine, Epstein-Barr virus (EBV), human papillomaviruses (HPV), lentiviruses and the Allovirus TTV were detected in the umbilical blood, amniotic fluid or fetal tissues at the end of healthy pregnancies. Mechanisms: IgG transport mechanisms, transported maternal cells and the lipid rafts were shown to be vehicles of this virus transport.

Results: 1.) The first contact of the concept of the developing fetal organism with latent viruses can be the life-long carriage of these viruses upon birth. 2.) The developing fetal immune system might create immunotolerance to certain viral antigens depending on the fact, whether the viruses replicate or are only latently present in the fetal cells. This partial immune tolerance may impair the post partum immune response facilitating tumour formation in the affected individuals. 3.) Herpesviruses may activate endogenous retroviral genes in fetal cells, modifying their differentiation and surface properties. Alternatively the pathogenesis of autoimmune diseases might be initiated by these modifications. 4.) The viruses are coding for micro RNA molecules possibly influencing the replication and differentiation of the virus carrier cells. The genes of DNA viruses interfering with apoptotic mechanisms may also disturb the normal differentiation processes in the organs of the fetus. 5.) Finally the response to the mandatory antiviral vaccinations might be impaired by the transplacently transferred viruses.

Conclusion: The systematic testing of umbilical blood and urine of newborn babies would be of essential importance using molecular techniques for the presence of the viruses in order to be prepared for yet unknown risks for the future life span.
Lacosamide is a Novel Antinociceptive and Antiepileptic Drug with a Dual Mode of Action

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Background: Lacosamide is an investigational drug that has demonstrated positive results in Phase III trials of neuropathic pain and epilepsy. Preclinical studies have shown neuroprotective effects of lacosamide both in animal models and in vitro. Electrophysiology and proteomics experiments have identified two likely modes of action for lacosamide.

Methods and Results: Electrophysiology experiments performed in mouse neoblastoma cells indicate that lacosamide reduces sodium-channel availability by selectively enhancing slow-inactivation. Enhancing slow-inactivation is thought to raise channel-activation thresholds, reducing pathological neuromodulation in nociceptive and antiepileptic agents, which non-selectively block the sodium channel pore and/or enhance fast- and slow-channel inactivation.

A second mechanism of action may occur via the binding of lacosamide to collagenase mediator protein 2 (CRMP-2), a phosphoprotein that is involved in neuronal differentiation and axonal out-growth (processes that are maladaptive in the pathophysiology of pain and epilepsy). The interaction of lacosamide with CRMP-2 may mediate the apparent neuroprotective effects of lacosamide, since CRMP-2 appears to be important for mediating neuroprotection from excitotoxic insult and apoptosis.

Conclusions: The dual mode of action of lacosamide represents two novel mechanisms for the treatment of nociception and epilepsy. Based on current studies, it is proposed that selective enhancement of slow-inactivation of sodium channels may underlie the immediate effects of lacosamide. Further characterization of the interaction with CRMP-2 may help to explain its role in lacosamide's symptomatic and disease-modifying effects.

Development of Unique Cisplatin Analogs for Site-Specific Treatment of Hormone-Dependent Female Cancers

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Background: Chemotherapy remains, to this day, an effective treatment for several types of cancer. However, the severe side effects caused by the treatment limits its full potential for a cure. Thus, the development of site-specific anticancer therapy is a subject of intense research. Several strategies can be used to target cancer cells. For instance, the use of a carrier molecule being able to recognise a specific receptor in the cell is a tactic of choice used by several research groups. We have developed several estradiol-platinum(II) (E2-Pt(II)) hybrid molecules using the following guiding principles: a) potential for affinity towards the estrogen receptor b) potential for in vitro and in vivo selectivity on hormone-dependent female cancers c) ease of synthesis and d) potential for large scale industrial production.

Methods: The development of the E2-Pt(II) hybrid molecules will be initially discussed. Then, the most promising hybrid derivative, VP-128, is selected to examine its biological activity towards breast (MCF-7, ZR-75-1, MDA-MB-468, MDA-MB-231 and HS578-T) and ovarian (OVCAR-3, SKOV-3, A2780 and A2780-Drk) cancer cells, in vitro (MTT assays) and in vivo (xenografts model) using ERα-positive or negative cells.

Results: MTT assays revealed that VP-128 decreased the viability of breast and ovarian cancer cells more efficiently than cisplatin itself in vitro. Moreover, in the case of breast cancer the expression of ERα sensitised the cells to the growth-suppressive effect of VP-128. Hoechst nuclear staining revealed an improved efficiency of VP-128 compared to cisplatin to induce apoptosis of breast cancer cells, which was enhanced in ERα-positive cells. In cisplatin resistant A2780-Dk cells, VP-128 was able to induce cell death indicating that the new drug might also be efficient to kill cisplatin resistant cancers. Finally, using human breast and ovarian cancer cell xenografts in nude mice, we found that VP-128 had stronger antitumour activity compared to cisplatin in vivo, and was more specific and selective towards hormone-dependent cancer cells.

Conclusions: Experimental data show that VP-128 possesses enhanced anticancer activity compared to cisplatin and is able to specifically target hormone-dependent tumours in an in vivo model. Thus ultimately, VP-128 could provide new and/or alternative treatment modalities for breast and ovarian cancers.

Mediterranean spotted fever (MSF) in Oran (Algeria)

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Abstract

MSF due to Rickettsia conori was thought for many years to be the only tick-borne rickettsial disease prevalent in Algeria. However, in recent years, other species within the spotted fever group of the genus Rickettsia have been described as emerging pathogens. Tick-borne agents include: Rickettsia aeschlimanii and Rickettsia massiliae. Many rickettsiae of unknown pathogenicity have also been detected from ticks and could represent potential emerging pathogens to be discovered in the future. Furthermore, a new spotted fever rickettsial, Rickettsia felis, was found to be associated with cat fleas and is an emerging human pathogen. Rickettsia felis is susceptible to doxycycline, thiamphenicol and fluoroquinolones but not to gentamicin, erythromycin, amoxicillin or trimethoprim-sulfamethoxazole. The resistance of this new species to erythromycin is consistent with a taxonomic position within the spotted fever group. We present an overview of these rickettsial species, focusing on emerging diseases.
Mechanisms that Explain the Lower Incidence of Breast Cancer in Postmenopausal Women Treated with Conjugated Estrogens (CEE). Role of Estrogen Receptor α and β.

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2Novus International Inc.

Background: Recent findings from the Women’s Health Estrogen Alone Trial (JAMA 2008), showed that long term treatment of hysterectomized postmenopausal women with CEE not only did not increase the incidence of breast cancer, but more importantly, may have reduced the risk in these women for this disease. These results raise some important questions as to the mechanisms involved and whether all types of estrogen can impart this protection. In the present study, the relative binding affinities (RBAs) of 11 equine estrogens and their functional activities mediated via ERα and ERβ were compared.

Methods: The RBAs were estimated by competitive binding assays using [14C]-estradiol and unlabelled equine estrogens. The functional activity of the estrogens was measured in HepG2 cells transfected with human ERα or ERβ or both and secreted-alkaline phosphalase (SEAP) gene and analyzed by a chemiluminescent assay.

Results: In comparison to 17β-estradiol (17β-E2), the RBAs of most ring B unsaturated estrogens were 2-8 fold lower for ERα and ERβ, however, these unique estrogens had 2-4 times greater affinity for ERβ than for ERα. The transcriptional activity of these 11 estrogens showed that all estrogens were functionally active. 17α-estradiol induced the activity of SEAP by ERα to a higher level than any other estrogen. Activity of other estrogens was 12% to 17% that of 17β-E2. In contrast, 17β-E2 stimulated the activity of ERβ to a 5 fold lower level than ERα. The activities of other estrogens mediated via ERβ were 66-280% that of 17β-E2 with equilenin being the most active. Except for 17β-E2, no correlation was observed between functional activities and the RBA’s for ERs. The activity of the ring B unsaturated estrogen components of CEE appear to be exerted predominantly through ERβ. To our knowledge, these are first such observations. Moreover, depending on the estrogen, ERα can act as a dominant repressor or dominant activator of ERβ’s transcriptional activity.

Conclusions: Taken together, these data indicate that all estrogens are not the same and have different pharmacology. Some ring B unsaturated estrogen components of CEE can via ERβ inhibit the proliferative effects of 17α-estradiol mediated via ERα, thereby reduce the risk of breast cancer in women who are just taking equine estrogens alone.

Nanodevices for targeted delivery: An evaluation of toxicological models

BHOagal N

FRAME, Russell & Burch House, 96-98 North Sherwood Street, Nottingham, UK

Background: Engineered nanoparticles and liposomal delivery systems (nanodevices) are increasingly utilized as a means of overcoming problems with the bioavailability, stability and toxicity of pharmaceuticals. Such systems promise to revolutionize clinical management and to obviate the need for lifestyle-based self-administration.

Methods: Some of the major challenges are described and the prospects for improving and stratifying toxicological assessment of nanodevices are considered. The importance of selecting the most appropriate toxicological models for biocompatibility testing is highlighted by the surfeit of new nanodevice-medicine combinations currently in the preclinical pipeline. The structures of these drugs are confirmed by nuclear magnetic resonance (NMR) spectroscopic methods.

Conclusions: In summation, highly diastereoselective synthesis of tamoxifen, toremifene, and droloxifene is achieved using organoborane reagents. Our current methods are compared with the literature methods to prepare these drugs.

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Conclusions: In summation, highly diastereoselective synthesis of tamoxifen, toremifene, and droloxifene is achieved using organoborane reagents. Our current methods are compared with the literature methods to prepare these drugs.
New derivatives of BM 212 with improved antimycobacterial activity.

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Background: According to the report 2007, compiled by the World Health Organization (WHO), the total number of new cases of Tuberculosis (TB) worldwide in 2005 had risen to approximately 8.8 million and there were approximately 1.6 million TB deaths during the same year. Increased infection with the M. avium complex (MAC) is also contributing to the morbidity and mortality in AIDS patients. The most urgent goal of chemotherapy of tuberculosis infections should be the development of highly active and low-cost drugs, which should be used not only in industrialized countries but also in developing ones in which both these infections are now rapidly increasing. As active molecules already introduced in therapy very soon generate resistance, scientists have focused their attention on the development of new antimycobacterial compounds acting with a mode of action without cross-resistance.

Methods: Many pyrrole derivatives, analogues of BM 212, have been synthesized on the basis of previous results and molecular modelling considerations based on the pharmacophore model previously identified for them. All the derivatives were tested for their cytoxicity and in vitro activity against many strains of M. tuberculosis, atypical mycobacteria, drug-resistant mycobacteria of clinical origin and intracellular mycobacteria. Protection Index (PI) was calculated and for the most active of them the bioavailability was also evaluated. The in vivo tests and the study of the mode of action are currently under study.

Results: Some of the synthesized compounds revealed more active than BM 212 against mycobacteria. In particular the PI for many of them was comparable to that of reference compounds, Isoniazid, Streptomycin and Rifampin. Many of the synthesized compounds revealed also to be active against intracellular mycobacteria and they showed to inhibit drug-resistant mycobacteria of clinical origin.

Conclusions: On the basis of our previous studies and molecular modelling considerations, many new derivatives of BM 212 were identified. In particular some of them revealed active and low toxic so that they can be considered very promising for future studies.

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Liquid Chromatography and Capillary Electrophoresis as a Tools to Study Ligand-Receptor Interactions

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The phenomenon responsible for enantioseparation in chromatographic and electrophoretic methods is the same; it is the enantioselective interaction between the enantiomers and a chiral selector. The principal difference between these two techniques arises from different separation process mechanisms and sometimes different environments of complexation. The Liquid Chromatography and Capillary Electrophoresis (CE) for enantioseparation has been used to advantage in many chromatographic and electrophoretic applications.

The chromatographic and electrophoretic methods which are very sensitive to structure, size, shape and dynamics of the analyte have been used not only in separation science but also in the study of molecular recognition processes. The analytical applications of basic chiral pharmaceuticals such as pheniramine, brompheniramine, metoxyphenamine, cyclopentolate, doxylamine, and ketamine was investigated in capillary electrophoresis (CE) and liquid chromatography (HPLC) using negatively charged sulfated β-cyclodextrin (β-CD) and neutral cyclodextrins (CDs). The apparent stability constants for the model compounds with cyclodextrins in both techniques were estimated.

Both methods seem to be complementary for the study of complexation phenomena. It can be seen that brompheniramine forms stronger complexes with β-CD than pheniramine and doxylamine. Complexation of pheniramine and doxylamine by β-CD is very similar. The weakest complexes β-CD forms with metoxyphenamine. For the studied compounds, TM-β-CD forms very weak complexes. The stability constant for DM-β-CD is very similar to that obtained for β-CD. From the native CDs the best chiral selectors for the studied compounds are β-CD and HP-β-CD.

For the studied compounds the best recognition between enantiomers was obtained for cyclopentolate (K_Wulk=1.32, K_Rulk=1.45 and K_Wulk=K_Rulk=1.26 for β-CD, for HP-β-CD and TM-β-CD, respectively) as the CE is the more efficient method, chiral recognition is better visible in this method than in HPLC.

Conclusions: The obtained results show that chromatographic and electrophoretic methods may be used as additional tools for studying weak interactions responsible for molecular recognition between ligand and receptor.
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Background: The urinary recovery of orally ingested solutions containing Mannitol (mol radius 4 Å) and Lactulose or Cr-EDTA (mol. radii 5 Å) is a widely used clinical test for small intestinal barrier function. In humans, recovery of Lactulose is < 0.5 %, of Mannitol < 20 %, giving a L/M recovery ratio of 0.025, which is very low compared to theoretically expected L/M diffusion ratios of 0.8 through aqueous pores Aims: To investigate the underlying mechanisms of this discrepancy by comparison of in vivo and in vitro probe permeability.

Methods: 1) Small intestinal sheets from rodents (rats, guinea pigs, rabbits) and human intestinal biopsies were mounted in Ussing chambers and mucosa-to-serosa fluxes of L/M were determined (n = 4-8). Urinary recovery of orally applied probes was measured in rodents, cats and humans (n = 2-6). 2) Absorption of Cr-EDTA, Mannitol and water was studied in situ perfused jejunal loops in anesthetized cats (n = 5), using four isotopic perfusion solutions with varying contents of NaCl and glucose.

Results: L/M flux ratios in vitro were about 0.8 in all tested species (0.68 to 0.98). Urinary recovery of L/M ratios in rodents ranged from 0.1 to 0.49. L/M ratios in cats and humans were 0.03 and 0.02, due to high mannitol recovery, resp. 29 and 22 %. In situ perfused cat jejunum there was a strong positive correlation between water absorption and mannitol clearance (r = 0.98, p < 0.003), no correlation between water absorption and Cr-EDTA clearance (r = 0.05, p = 0.95). Likewise, there was a lack of correlation between water absorption and Cr-EDTA/Mannitol ratios (r = 0.98, p < 0.002).

Conclusions: Interspecies variation in urinary recovery of mannitol is caused by differences specific for the gut mucosa barrier to avoid competition of villus tips in vivo varying, being highest in humans and cats as a result of efficient vascular countercurrent multiplication because of their villus vascular anatomical structure. Thus we hypothesize that the high mannitol recovery in both species is caused by solvent drag through pores that allow the passage of Mannitol, but not vascular countercurrent multiplication. Mannitol clearance in cat jejunum in different species with varying solutions which differently affect the capability of the countercurrent multiplier mechanism confirms this hypothesis.

Mannitol, A Key Probe Molecule in the Assessment of Small Intestinal Permeability?

Bilensoy E1


Background: Paclitaxel is a potent anticancer drug associated with severe side effects due to the use of co- solvent Cremophor in its commercial injectable formulation. It is known that nanoparticulate drug delivery systems can provide an alternative for the effective delivery of anticancer agents due to the EPR effect facilitating the targeting of the encapsulated anticancer drug to tumor cells instead of healthy tissues. Objectives of this study was to develop a nanoparticulate carrier system for paclitaxel with high encapsulation efficiency, controlled drug release property which may be more advantageous than cremophor vehicle in terms of safety and efficacy.

Methods: Nanoparticulates of amphotericil (3-cyclodextrin loaded with paclitaxel were prepared using nanoprecipitation technique. Particle size distribution and morphological features of the nanoparticles were studied. The nanoparticles were evaluated for their in vitro drug release profiles with HPLC assay. Safety of the nanoparticles in vitro (nanocapsules and nanospheres) were assessed in terms of both hemolysis and cytotoxicity to L929 cells in comparison to commercial injectable formulation. Placement efficacy of paclitaxel loaded nanocapsules and nanospheres were evaluated by MTX assay against human breast cancer cell line MCF7 with MTT assay. Results: Size for nanocapsules and nanospheres were found to be around 350 nm and 180 nm respectively and were found to be stable for a storage period of 12 months as seen in Figure 1. Encapsulation efficiency was increased by 2 to 3 fold by incorporating paclitaxel into cyclodextrin nanoparticles. The drug was released within 6 hours. Hemolytic order was found for Cremophor vehicle>nanocapsules>nanospheres. Ethynycthols were imaged by SEM after treatment with paclitaxel loaded formulations. Cytotoxicity of blank nanoparticles were significantly lower than cremophor commercial vehicle as paclitaxel demonstrated equal anticancer efficacy in nanoparticles.

Conclusions: Amphotericil cyclodextrin nanoparticles can be considered as an alternative dosage form for injectable paclitaxel in terms of safety and efficacy. Analysis of drug-receptor interaction at equilibrium

Bindslev N

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Aim: We want to analyze our dose-response data at equilibrium by models. We may have an idea about a possible physical interpretation of obtained response curves and use either mathematical or mechanistic descriptions.

Background: From 1901 to 1910, receptors as separate entities were recognized by Eliot (1904) and Langley (1905) in Cambridge and Ehrlich (Frankfurt 1907). Deviation from a simple Langmuirian hyperbola (1918) was documented by C. V. Schramm (Copenhagen 1940) for oxygen-hemoglobin binding.

Mono-ligand systems: For mono-ligand systems with dose-responses deviating from a simple hyperbole, Hill-s equation can give you a quantitative measure of cooperativity; but without any information about ligand interactions. Therefore, co-operative values from a Hill scheme most likely has no physical correlate. In a physical description of systems deviating from simple hyperbolism, I suggest the homotropic two-state model, HOTS (2004). This model can handle both positive and negative co-operativity as well as bell-shaped and reverse bell-shaped relationships.

Two-ligand systems: Models for two ligands may again be based on either a non-interaction/-interaction scheme such as the non-competitive inhibition scheme without two-states of the un-ligated receptor or on an extended Monod-Wyman-Changeux scheme (Monod et al 1965, Rubin & Changeux 1969) with two-states. Both these approaches assume no interaction between binding sites, i.e., upon binding of a ligand there is no change in the binding constant for a second drug.

Monod-Wyman-Changeux model: One of the first models to be developed for two ligands was the Monod-Wyman-Changeux model (1965). This model captures the effects of competition for binding sites and takes into account the allosteric effects of ligand binding. The Monod-Wyman-Changeux model assumes that the binding of a ligand to one site affects the binding of another ligand to a different site on the receptor.

Conclusions: Analyzing drug-receptor interactions at equilibrium using mathematical models such as the Hill equation or the Monod-Wyman-Changeux model provides valuable insights into the mechanisms of action of drugs and may help in the development of more effective therapeutic strategies.
Severe infections are a major cause of morbidity and mortality in immunocompromised patients. The most common fungal responsible for severe infections are Aspergillus. Voriconazole is a broad spectrum second generation triazole antifungal agent. It is indicated for the treatment of invasive aspergillosis. Pediatric dosage finding and safety evaluations have not been completed. Aims: 1) The aim of this study was to review the literature about studies done on the PK of voriconazole in children. 2) The clinical results of our 5 cases with Aspergilus infection (4 pulmonary, 1 bloodstream) with voriconazole treatment were shown.

Methods: Literature was searched with the key words “voriconazole, pharmacokinetics, children, invasive fungal infection” and the studies were evaluated.

Results: Children require higher doses of voriconazole than adults to attain similar serum concentrations over time because the drug exhibits nonlinear pharmacokinetics in adults, but exhibits linearity in children. A significant relationship between disease progression and drug concentration was described in adults (Antimicrob Agents Chemother 2006;50:1570). Based on studies in children, it appears that a pediatric dosage of 11 mg/kg administered every 12 h is approximately bioequivalent to an adult dosage of 4 mg/kg given 12 h. Plasma samples for voriconazole HPLC assay from 14 subjects revealed that in children receiving dosages of 34 mg/kg iv bid was lower than that of adult volunteers receiving 4 and 5 mg/kg iv bid (Walsh TJ, et al). In another study done in 5 children with ages ranged from 2 to 10 years old to test the hypothesis that TRPC channels had mutation frequency to cipr ofloxacin resistance. In nutritionally depleted medium No.1 it was only 57 fold rise. In cipr ofloxacin-resistant strains generated by H₂O₂ it was RI increasing up to 33-fold of spontaneous SOCE activity. This alternative hypothesis postulates Orai as the SOCE channel activated by STIM and does not include a role for TRPCs. However, as we published in Jan 2007, Orai and TRPCs interact functionally, as seen in a TRPC-dependent enhancement of SOCE upon expression of low levels of Orai. We proposed that instead of forming channels, Orai appear to be regulatory proteins that confer Ca²⁺-selective SOCE channel properties to the otherwise non-selective cation channels formed by TRPCs without Orai. In support, SOCE channels were shown in other laboratories to be dynamically assembled in lipid rafts from which TRPCs and Orai can be co-immunoprecipitated. A survey of the literature shows that TRPCs play a role in development that TRPCs play a role in development of the Development of the development of Magic Bullets that will help in better understanding their role and in ameliorating diseases involving altered TRPC and/or Orai functions.

Structure and Function of the Ubiquitous TRPC channels: Targets in Need of Novel Magic Bullets.

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The canonical transient receptor potential (TRPC) channels were discovered in our laboratory in 1995–96. They are homologues of the Drosophila light-activated and T-type like and were cloned to test the hypothesis that in mammalian cells they might be at the root of not only G protein-coupled-selective cation channels, but store-operated channels (SOCSE). In initial studies, 11 Orai 1 channels at high dose had a homology to voltage-gated cation channels and span the membrane 6 times. Between their discovery and now, the participation of TRPCs in being of interest for a large number of children. Voriconazole is a broad spectrum second generation triazole antifungal agent. It is indicated for the treatment of invasive aspergillosis (that generates adenosine) and Akinledorethral nitric oxide synthase (eNOS) with downstream activation of inducible NO synthase (iNOS) and COX2 inhibition of the PI3K, adenosine receptors, eNOS, iNOS and COX2 abrogates the IS-limiting effects of statins.

Methods: Rats received 3-day oral atorvastatin (ATV) or vehicle with or without dipyridamole (DIP, 6mg/kg/d), cilostazol (CIL, 20mg/kg/d), or aspirin (ASA, 5, 10 or 20mg/kg at reperfusion). Rats underwent 30-minute coronary artery occlusion and 4-hour reperfusion.

Results: ATV (10mg/kg/d) limited IS. Intravenous ASA before reperfusion attenuated this effect. DIP alone and ATV (2mg/kg/d) alone had no effect on IS; however, IS was significantly reduced in the ATV+DIP combination. Myocardial adenosine levels were higher in the ATV+DIP group than in the ATV alone, DIP alone and the control group. The protective effect was abolished with theophylline, indicating that it is mediated by adenosine receptor activation. CIL alone, and especially when combined with ATV (2mg/kg/d) limited IS. CIL increased myocardial levels of adenosine and Akt and eNOS phosphorylation. In addition, by increasing tissue cAMP levels, CIL activated protein kinase-A that phosphorylates eNOS, CIL inhibited PTEN, thus leading to augmentation of Akt and subsequently eNOS phosphorylation. Aspirin blocks the IS-limiting effects of statins, whereas both dipyridamole and cilostazol have synergetic effects with statins. It might be that the anti-platelet regimens should be modified for patients receiving statins.

Conclusion: Starvation and Oxidative Stress as an Inducer of Ciprofloxacin Resistance

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Background: Mutation rate of bacteria is often affected by environmental conditions. Various stress such starvation, oxidative stress can result in increased frequency of mutations leading to antibiotic resistance. The aim of this work was to determine the mutation frequency leading to ciprofloxacin resistance induced by depleted media and hydrogen peroxide and to find molecular determinants of ciprofloxacin resistance in selected mutants.

Methods: Oxidative stress was induced by 3h cultivation of S. typhimurium with hydrogen peroxide in 3 concentrations (0.4, 0.8, 2, 4 mM). Starvation was studied after incubation (3h) of bacteria in control Luria-Bertani medium (LB) and in nutritionally depleted media 10%LB and Nutrient broth No.1. Resistant strains were counted on agar plates supplemented with ciprofloxacin (0.06 mg/ml) after 72 h of incubation at 37°C. The frequency of resistant mutants (resistance index RI) represents mean number of resistant cells divided by the total number of viable cells per culture. Data represent the mean of three independent experiments; each experiment was made in five parallels and statistically evaluated by Student's t-test. Mutations in gyrA were determined using ASc-PCR-RFLP method. Levels of outer membrane porin F were detected with SDS-PAGE.

Results: Short-term cultivation in 10% LB caused 430 fold increase of RI while in Nutrient broth No.1 it was only 57 fold rise. In ciprofloxacin-resistant strains generated by long-term starvation were detected decreased levels of OmpF protein. With rising dose of H₂O₂ was RI increasing up to 33-fold of spontaneous mutation frequency to ciprofloxacin resistance. In nutritionally depleted medium with H₂O₂ has mutation frequency increased more than 103-time. 80% of resistant strains had mutation in gyrA. 37% of them had mutation in ccdon Ser-83 and 63% in ccdon Asp-87.

Conclusions: 1) Starvation increases mutagenesis leading to ciprofloxacin resistance. 2) Short-term treatment of S. typhimurium in nutritionally depleted media caused higher increase of ciprofloxacin RI than long-term incubation. 3) Long-term starvation is leading to decreased levels of OmpF. 4) Oxidative stress induced by H₂O₂ in conjunction with lack of nutrients in environment increases mutation frequency to ciprofloxacin resistance. 5) Majority of ciprofloxacin-resistant strains generated by H₂O₂ has mutation in gyrA gene.

This work was supported by the Slovak Grant Agency VEGA (Projects no.14305/07)
Identification of the anti-inflammatory targets of interactive constituents of *Hypericum perforatum* (Hp).

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Background: Hp is used as a botanical therapy for infective disorders. The level of constituents in Hp extracts were lower than the concentration of pure constituents needed to reduce lipopolysaccharide (LPS)-induced prostaglandin E2 (PGE2) production in RAW 264.7 macrophages, suggesting an interaction of compounds for the activity. The goal of this study was to identify key constituents and investigate the gene targets for the anti-inflammatory effect.

Methods: A flavonoid-rich bioactivity guided fractionation was used with screening in the LPS-induced RAW 264.7 macrophage system to identify active fractions from each round and liquid chromatography-mass spectrometry (LC-MS) identified constituents present. CellTiter96TM Aqueous solution revealed no significant cytotoxicity with fractions or constituents at the doses studied. Microarray analysis was performed with Hp fraction, the 4 constituents combined at levels detected in the Hp fraction, and control solvent without LPS.

Results: A third round fraction (3A) (10 μg/ml) significantly reduced PGE2. Combining four constituents at concentrations detected in the LC-MS analysis (0.17 μM chlorogenic acid, 0.08 μM amentoflavone, 0.08 μM quercetin, and 0.03 μM pseudohyperin (PHCN)) explained the anti-inflammatory activity of the fraction in light-activated conditions. The amount of each pure constituent needed to observe a significant reduction in PGE2 > 50 times more than was found in fraction 3A. Of the 4 interacting compounds, only PHCN was required. With LPS, the 4 component system affected 162 genes and the fraction affected 780 genes; 40 genes were differentially expressed under both treatments. Important pathways for both treatments were the Janus kinase-signal transducer and activator of transcription (JAK-STAT) and the glycoprotein (EGP), glucose infusion rate (GIR), glucose disposal rate (GDR) and skeletal muscle glucose utilization index (GUI). Moreover, the status of oxidative stress as reflected by urinary levels of iso prostane and tissue contents of protein-bound carbonyls and thiobarbituric acid reactive substrates (TBARS) were also assessed by and approximate 6 months after therapy. While differences in protein profiles were minor for early-stage disease differences became significant for more advanced stage tumors. Follow up of patients over multiple years showed that certain biomarker candidates changed in agreement with the recurrence of disease. Some of these candidates were related to the glycosylation of serum proteins.

Conclusions: 1) Biomarker candidates have been discovered that correlate with the response to therapy for cervical cancer. 2) Dedicated data processing and statistical analysis reduced the number of variables to a number that is in-line with the number of analyzed samples.


Synthesis of Unnatural Ceramide Analogues and Their Antiproliferative Properties Against a Panel of Cancer Cells

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Abstract: 23,3R-Ceramide 1 occupies the “hub” of sphingolipid metabolism and serves as a coordinator of eukaryotic stress responses and other biological activities such as cell growth and differentiation. Ceramide 1 plays a key role in programmed cell death (apoptosis). However, since 1 is a naturally occurring lipid, it is recognized by endogenous enzymes and can be converted into anti-apoptotic lipids via phosphorylation and acetylation. We sought to prepare unnatural ceramide analogues that may be longer lived in cells because they are unrecognized by the enzymes. We found that some of the unnatural ceramide analogues have greater antiproliferative activity than 1 against human breast cancer cell lines in vitro. One of the unnatural ceramides analogs we synthesized has an exocyclic double bond at the sphingoid base (compound 2), whereas another has a disulfide linkage in the N-acyl chain (compound 3). Their antiproliferative activities against three human breast cancer cell lines (BT466, MDA-MB-231, MCF-7), a lung cancer cell line (A549), a prostate cancer cell line (DU145), and a cervical cell line (HeLa) were analyzed in vitro and compared with the activity of (23,3R)-N-octanoylceramide (4). The sulfur-containing-ceramide analogue 3 and the acyl-ceramide 2 exhibited a higher antiproliferative activity than natural ceramide 1. Caspases in cells treated with compound 3 were activated, indicating that the cells underwent apoptosis. A ceramide analog containing a tetrathydrofuranyl ring (compound 4) and a phytoceramide analog (compound 5) exhibited a much higher antiproliferative activity than natural ceramide 1. Caspases in cells treated with compound 3 were activated, indicating that the cells underwent apoptosis. A ceramide analog containing a tetrathydrofuranyl ring (compound 4) and a phytoceramide analog (compound 5) exhibited a much higher antiproliferative activity than natural ceramide 1. Caspases in cells treated with compound 3 were activated, indicating that the cells underwent apoptosis.
Background: About 80% of people in the developing world, particularly those from rural communities where modern drugs are unavailable, inaccessible or, unavailable, rely on phytotherapy for primary healthcare. However, most medical and veterinary professionals distrust herbal medicines due to lack of scientific evidence and safety. Hence, there is need for their validation, before herbal medicines gain wider acceptance and use. Traditional healers, pastoralists and rural farmers use extracts of Peltophorum africanum (a medicinal plant widely spread in southern Africa and other tropical regions), to treat diarrhea, dysentery, pain, infertility, HIV/AIDS and to promote well-being and resistance to diseases. The extracts of the plant inhibit HIV-1 reverse transcriptase and protease.

Methodology: Dried leaves and bark from mature P. africanum trees were extracted with acetone. Chromatograms were made on silica gel plates. Minimum inhibitory concentrations (MIC) values of 0.08 mg/ml for Staphylococcus aureus and 0.16 mg/ml for Pseudomonas aeruginosa. The extracts showed higher antifungal activity than amphotelin B. The acetone extracts of the bark, and root of P. africanum showed higher antioxidant activity than L-ascorbic acid (Vitamin-C) and much higher than Gastrointestinal nematodes exasperate diarrhea in HIV-AIDS patients, as well as disease-related host. The traditional use of antioxidants in the control of neurological diseases by either directly inhibiting bacterial growth or by stimulating the immune system of the host. The traditional use of antioxidants for primary healthcare. However, most medical and veterinary professionals distrust herbal medicines due to lack of scientific evidence and safety. Hence, there is need for their validation, before herbal medicines gain wider acceptance and use. Traditional healers, pastoralists and rural farmers use extracts of Peltophorum africanum (a medicinal plant widely spread in southern Africa and other tropical regions), to treat diarrhea, dysentery, pain, infertility, HIV/AIDS and to promote well-being and resistance to diseases. The extracts of the plant inhibit HIV-1 reverse transcriptase and protease.

Results: The extracts showed antifungal activity against both Gram-positive and Gram-negative bacteria, with Minimum Inhibitory Concentration (MIC) values of 0.08 mg/ml for Staphylococcus aureus and 0.16 mg/ml for Pseudomonas aeruginosa. The extracts showed higher antifungal activity than amphotelin B. The acetone extracts of the bark, and root of P. africanum showed higher antioxidant activity than L-ascorbic acid (Vitamin-C) and much higher than Gastrointestinal nematodes exasperate diarrhea in HIV-AIDS patients, as well as disease-related host. The traditional use of antioxidants in the control of neurological diseases by either directly inhibiting bacterial growth or by stimulating the immune system of the host. The traditional use of antioxidants for primary healthcare. However, most medical and veterinary professionals distrust herbal medicines due to lack of scientific evidence and safety. Hence, there is need for their validation, before herbal medicines gain wider acceptance and use. Traditional healers, pastoralists and rural farmers use extracts of Peltophorum africanum (a medicinal plant widely spread in southern Africa and other tropical regions), to treat diarrhea, dysentery, pain, infertility, HIV/AIDS and to promote well-being and resistance to diseases. The extracts of the plant inhibit HIV-1 reverse transcriptase and protease.

Conclusion: P. africanum extracts have therefore, potential for treatment of infection-related diseases by either directly inhibiting bacterial growth or by stimulating the immune system of the host. The traditional use of P. africanum concoctions against diarrhea, dysentery and unthriftiness, may be improved if these extracts are confirmed with parasitic gastrointestinal. Gastrintestinal nematodes exasperate diarrhea in HIV-AIDS patients, as well as disease-related production loss arising from stock mortality, severe weight loss and poor production in ruminants. Antioxidants are also important in boosting the immunity, critical in the management of immunosuppression. There is ample scientific and empirical evidence suggesting the use of plant-derived antioxidants in the control of neurological diseases, as antioxidants have neuro-protective (presynaptic and postsynaptic) and neuro-regenerative roles. Due to the high antioxidant activity of its extracts, P. africanum has prospected in the management or control of neurodegenerative diseases. Thus there is great potential of P. africanum extracts in disease control.

Development of a new 3D-Human Airway Epithelium/Whole-blood Co-culture Model Combined with Multi-Analyte Profile (MAP) Analyses for Assessing Drug Effects

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Background: The dialogue between cells of the immune system and cells of various tissues controls immune reactions and is in part mediated by a variety of immunomodulatory para-noxae. Our recent results have shown that the oxysterol 24OHC may efficiently pass a model for the blood-brain barrier. By measuring the concentration difference between the internal jugular vein and an artery in human volunteers, we demonstrated the production of 24OHC by the brain to be about 6 mg/24h. Since there is a good correlation between levels of cholesterol and 24OHC in the circulation, it seems likely that the uptake of 24OHC by the brain is related to the levels of cholesterol in the circulation. In spite of the fact that cholesterol does not pass the blood-brain barrier, hypercholesterolemia is a risk factor for Alzheimer Disease (AD). We hypothesized that there may be a metabolite of cholesterol fluxing in the opposite direction from the circulation into the brain. This metabolite was identified as 27-hydroxycholesterol (27OHC), also using the catheterization approach. The uptake of this oxysterol by the human brain was found to be about 5 mg/24h. Since there is a good correlation between levels of cholesterol and 24OHC in the circulation, it seems likely that the uptake of 27OHC by the brain is related to the levels of cholesterol in the circulation. In spite of the relatively high influx, levels of 27OHC in the brain are very low, indicating an efficient metabolism. The major metabolite was identified as 1,27-dihydroxy-3,4-cholestanol. This acid very efficiently passed a model for the blood-brain barrier and we found a net flux of it from the human brain into the circulation. The conversion of 27OHC into the steroid acid can be regarded as a regulated modification. 27OHC is an efficient suppressor of cholesterol synthesis and we have shown that the compound is able to increase amyloid formation in neuroblastoma cells.

Therapeutic Development of Insulina Cell Lines Treated with Streptozotocin

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Background: Streptozotocin (STZ) is a member of a group of alkylating antineoplastic drugs, and is clinically active against insulinas. STZ toxicity depends on glucose transporter protein-2 (GLUT-2) expression and generation of free radicals. As with many other chemotherapeutic drugs, repeated treatment with STZ, may induce a selection of resistant cell populations. Differentiation therapies are seen as a promising approach to selectively engage the process of tumor cell differentiation during chemotherapy. According to this approach, cytotoxic agents can induce drug resistance, but in certain conditions, can also lead to recovery of normal cell homeostasis. Aim: To estimate tumor resistance and differentiation of insulina cell survival following exposure to STZ. Methods: Immunophenotypical studies in rat insulinoma cell-line (BTC-T) and low differentiated rat insulina cell-line (RINm5F) were repeated exposed to STZ.

Results: The cell populations (RINm5F and BTC-T) surviving such treatment were examined by immunophenotypical studies in rat insulinoma cell-line (BTC-T) and low differentiated rat insulina cell-line (RINm5F) were repeated exposed to STZ.

Conclusions: The STZ treatment of parental insulina cell lines resulted in the selection of resistant tumor cell-line to different toxins. The enhanced tumor tolerance may be explained by a low level of GLUT-2 and high expression of GLUT-2 in selected cells. In addition, STZ selected cells displayed a lower rate of cell proliferation when compared to untreated cells. Moreover, BTC-T and RINm5F sub-lines showed a 2.5 times higher level of intracellular insulin content in comparison to BTC-T. In order to test the sensitivity of rat insulinoma cell lines to STZ, the insulinoma cell lines were treated with STZ, and the intracellular insulin content was determined. The Western blot analysis was applied to estimate GLUT-2 and GLUT-2 expression in parental and STZ selected cells. Insulin content and secretion, cell proliferation, morphology and characteristics were studied.

Results: Repeated STZ treatment of parental insulina cell lines resulted in the selection of resistant tumor cell-line to different toxins. The enhanced tumor tolerance may be explained by a low level of GLUT-2 and high expression of GLUT-2 in selected cells. In addition, STZ selected cells displayed a lower rate of cell proliferation when compared to untreated cells. Moreover, BTC-T and RINm5F sub-lines showed a 2.5 times higher level of intracellular insulin content in comparison to BTC-T. In order to test the sensitivity of rat insulinoma cell lines to STZ, the insulinoma cell lines were treated with STZ, and the intracellular insulin content was determined. The Western blot analysis was applied to estimate GLUT-2 and GLUT-2 expression in parental and STZ selected cells. Insulin content and secretion, cell proliferation, morphology and characteristics were studied.

Conclusions: Repeated STZ treatment of insulinoma cell lines resulted in the selection of cell sub-populations possessing multiple toxin resistance, a low rate of proliferation and enhanced function. Further functionalization of STZ selected beta cells could provide useful lessons for optimization of differentiation therapy of cancer.

Abstracts

EHLRICH II –2nd World Conference on Magic Bullets

Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich

Nürnberg, October 3-5, 2008

Brain cholesterol? Long secret life behind a barrier

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The blood-brain barrier is almost completely impermeable for cholesterol in both directions. All cholesterol present in the brain is thus a product a local synthesis. Since there is a low but significant synthesis of cholesterol in the adult mammalian brain, we hypothesized that there may be a compensatory flux of a cholesterol metabolite from the brain across the blood-brain barrier. About 14 years ago we identified this metabolite as 24S-hydroxycholesterol (24OHC) and showed that in contrast to cholesterol itself, this oxysterol can cross the blood-brain barrier. By measuring the concentration difference between the internal jugular vein and an artery in human volunteers, we demonstrated the production of 24OHC by the brain to be about 6 mg/24h. Since there is a good correlation between levels of cholesterol and 24OHC in the circulation, it seems likely that the uptake of 24OHC by the brain is related to the levels of cholesterol in the circulation. In spite of the relatively high influx, levels of 27OHC in the brain are very low, indicating an efficient metabolism. The major metabolite was identified as 1,27-dihydroxy-3,4-cholestanol. This acid very efficiently passed a model for the blood-brain barrier and we found a net flux of it from the human brain into the circulation. The conversion of 27OHC into the steroid acid can be regarded as a regulated modification. 27OHC is an efficient suppressor of cholesterol synthesis and we have shown that the compound is able to increase amyloid formation in neuroblastoma cells.

Different pathogenetic aspects of the oxysterol crosstalk over the blood-brain barrier will be discussed in the lecture and it is suggested that the flux of 27OHC from the circulation into the brain is the missing link between hypercholesterolemia and AD.

All abstracts are listed in alphabetical order of the presenting author.
Drug Potential of Nigerian Medicinal Related Plants: A SURVEY

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Medicinal plants are plants used whole or parts to prevent and cure health problems, promote and rehabilitate nature to the living population at primary, secondary and tertiary health care deliveries. In Nigeria, the use of plants at various levels of health care delivery has been in practice for many centuries past; as old as the history of human beings. These plants belong to several families including Leguminosae, Malvaceae, Mimosaceae, Euphorbiaceae, Compositae, Acanthaceae, Cannaraceae, Passfloraceae, Rutaceae, Zingiberaceae, Bombacaceae, Otcaceae, Apocynaceae, Liliaceae, Sapindaceae and Combretaceae. Specific examples of the phyto-organisms are Pterocarpus, Phaetopus, Myrobolan, Plecoarpa, Picralima, Fig, Bihadram, Akabera, Mitteleif, Bear’s breathe, Copper leaf, Acalphya, Acacia, African mallow and Baobob.

Combretaceae. Specific examples of the phyto-organisms are Pericopsis, Bombacaceae, Olacaceae, Apocynaceae, Guttiferae, Liliaceae, Sapindaceae and Acanthaceae, C annaraceae, Passifloraceae, Rutaceae, Zingiberaceae, Combretaceae and others. Problems, promote and rehabilitate nature to the living population at primary, secondary and tertiary health care deliveries. In Nigeria, the use of plants at various levels of health care delivery has been in practice for many centuries past as old as the history of human beings. These plants belong to several families including Leguminosae, Malvaceae, Mimosaceae, Euphorbiaceae, Compositae, Acanthaceae, Cannaraceae, Passfloraceae, Rutaceae, Zingiberaceae, Bombacaceae, Otcaceae, Apocynaceae, Liliaceae, Sapindaceae and Combretaceae. Specific examples of the phyto-organisms are Pterocarpus, Phaetopus, Myrobolan, Plecoarpa, Picralima, Fig, Bihadram, Akabera, Mitteleif, Bear’s breathe, Copper leaf, Acalphya, Acacia, African mallow and Baobob.

Background: Drug-exciipients interactions attract research interest as a biopharmaceutical tool to influence the onset, intensity and the duration of performance in vivo. Aim: to improve the dissolution characteristics and optimize in vitro drug release profile of some non-steroidal anti-inflammatory drugs - indomethacin (IND), ibuprofen (IBP), naproxen (NAP), ibuprofen (IBP) by in vitro interactions with hydrophilic polymers - polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), hydroxyethyl cellulose (HEC), sodium alginate, dextran and silica-PPV or silica-methylnitroacetyl nanohybrids.

Methods: Different pharmaceutical techniques – solid dispersions, adsorbrates formation, sol-gel reactions and appropriate modern physico-chemical (FT-IR, X-ray, DSC, solid state C13 and D29 NMR, AFM, TEM) - and in silico methods for characterization have been applied.

Results: Changes in physico-chemical properties of drugs have been achieved - polymeric transitions (IND), inhibition of crystallization and amorphization (IND, IBP), complex formation (IND, IBP), particle size reduction. All changes are related to significant increase in in vitro drug dissolution. Special emphasis has been put upon the elucidation of the character of occurring interactions on molecular level. The modern sol-gel technique has been applied to develop silica-PVP-IBP model nanohybrids of prolonged, pH-independent IBP release. The main advantage of this reaction is that it takes place at mild experimental conditions and enables drug immobilization into the inorganic-organic network.

Conclusions: Physico-chemical transformations of some non-steroidal anti-inflammatory drugs by means of purposeful interactions with polymers and nanohybrids have been proven. Significant improvement of in vitro drug dissolution and modified drug release profiles were registered. The results give reasons to assume an increase of in vivo drug activity and safety.

The influence of cytarafine and myristic acid on aspirin binding with serum albumin. Spectroscopic study

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Background: In the body drugs are transported mostly via circulatory system as the complexes with albumin. This is an important part of the drug metabolism since the bound fraction of a drug has no pharmacological effect. The simultaneous binding of other exo- and endogenous ligands (such as drugs or fatty acids) can alter protein affinity towards drug and change the concentration of its free fraction. In our study the influence of cytarabine (araC) and myristic acid (MYR) on aspirin (ASA) binding with defatted bovine serum albumin (BSA) was investigated.

Methods: NMR spectra were recorded on Bruker Avance 400 spectrometer using 5 mm tubes. For water signal suppression the presaturation method was used. All solutions were prepared in D2O. Chemical shifts of proton resonances were calculated in relation to DSS signal (0.0 ppm).

Results: The association constants calculated for the first (subdomain IIA) and second (subdomain IIA) class of ASA binding site is 84.2x10^6 M^-1 and 1.81x10^7 M^-1, respectively.

Conclusions: The association constants calculated for the first (subdomain IIA) and second (subdomain IIA) class of ASA binding site is 84.2x10^6 M^-1 and 1.81x10^7 M^-1, respectively.

All abstracts are listed in alphabetical order of the presenting author.
Control of ceramide levels by ceramide kinase: evidence from knockout animals and use of a novel potent inhibitor


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Abstract: The sphingolipid ceramide is an important regulator of cell biology (e.g., apoptosis, differentiation) and its levels therefore must be tightly controlled. Ceramide kinase (Cerk) is a unique enzyme that specifically phosphorylates ceramide into the bioactive lipid ceramide-1-phosphate (C1P). Recent data from our laboratory have provided compelling evidence that Cerk is a key regulator of both C1P and ceramide levels.

Methods: Cerk-deficient (Cerk−/−) Balb/c mice were generated as described in Graf et al. J Immunol 2008, 180:3457-66, and compared to control littermates in all studies. The diaminobenzothiazole derivative NVP-231 was synthesized as described in Graf et al. Mol Pharmacol 2008 74(3), in press. Cerk activity assays using either fluorescently labeled ceramides or [32P]-ATP were described in Booth et al. J Biol Chem 2008 283:8517-26 and Rovina et al. Biochem J 2006 400:255-65, respectively. Growth and culture of COS-1 cells and bone marrow derived macrophages as well as Liquid Chromatography/Mass Spectrometry (LCMS) analysis was performed as described in Graf et al. J Immunol 2008, 180:3457-66.

Results: First, using a fluorescently labeled ceramide molecule to trace Cerk activity we found that C1P is short-lived compared to other ceramide metabolites, thus fulfilling an essential criteria for signaling function. This suggested that Cerk activity might be useful to disguise of excess ceramide. Then we proffited Cerk mice and we found indeed that ablation of Cerk not only decreases C1P levels but also leads to an increase in ceramide levels. Finally, we identified and characterized NVP-231, the first potent, specific and reversible Cerk inhibitor. Consistent with the observations in Cerk−/− mice, preliminary experiments with NVP-231 suggest that Cerk inhibition may have potential to regulate C1P and ceramide levels.

Conclusions: These results establish Cerk as a novel, key regulator of ceramide levels and support further exploration of Cerk inhibition as a rationale for the treatment of proliferative disorders.

References


Discovery of novel non-cyclam polyammoniated CXC4R4 coreceptor inhibitors


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Abstract: CXC4R4 and CCR5, chemokine receptors of the primary receptor (CD4) for the HIV cell fusion and entry, have been validated as targets for therapeutic intervention against AIDS. Bicyclams were the first non peptidic low molecular weight compounds with specific interaction with CXCR4 and the most potent bicyclam being AM3100 (IC50 > 10 ng/mL). However, it showed poor oral absorption and toxicity related to its high positive charge at physiological pH. To overcome such problem, we designed a combinatorial library of tetrakisramines 1, which preserve the main features of AM3100: a) at least two nitrogen atoms on each side of the p-phenylene moiety, one in the benzyllic position and the other(s) in a heterocyclic system and b) similar distances between such nitrogen atoms with those present in AM3100.

Methods: 19 compounds were initially selected by evaluating a series of molecular 2D and 3D descriptors. A PICA reduced the initial set of descriptors to 5 components which were used for the diversity selection. Anti-HIV activity (EC50) and cytotoxicity (CC50) measurements were carried out in MTT assays with HIV infected T4 cells. Results: The first subset of compounds showed EC50 in the range 0.8-18 µg/mL. A second subset of 17 compounds afforded 12 compounds presenting EC50 in the range 0.2-2.7 µg/mL. The third and final subset, covering up the total of 53 synthesized, was selected using QSAR techniques and ligand and structure-based drug design (using our CXC4R4 and CCR5 modeled receptors). Among them, 11(80%) showed an EC50 value of 0.008 µg/mL and a CC50 > 25 µg/mL, presenting nearly the same activity as AM3100 but showing no cell toxicity at tested concentrations. Conclusions: 1) A diversity oriented selection has allowed the synthesis of tetrakisramines 1 covering a broad range of activity values, useful for QSAR calculations. This approach afforded compound 11(80%), with an EC50 value of 0.008 µg/mL and a CC50 > 25 µg/mL. 2) Studies on the mode of action of compounds 1 showed specific inhibition of the CXC4R4 coreceptor.
Heparin as an inhibitor of cancer progression; the role of selectins

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Background: Heparin and low molecular weight heparin (LMWH) are widely used in cancer patients at risk of venous thromboembolism, which is a recognized complication of malignant disease. Recent clinical trials with LMWH and meta-analysis of earlier clinical trials with unfractionated heparin indicate that heparin affects cancer progression. Meanwhile, heparin and LMWH were repeatedly shown to reduce metastasis in a variety of animal models. Heparin is a natural product, comprising a polydisperse mixture of highly sulfated glycosaminoglycan chains, only a fraction of which bind antithrombin.

Methods: Experimental metastasis study was performed in wild type as well as in P- and/or L-selectin deficient mice. Treatment with heparin or their derivatives was performed only shortly before or after the application of tumor cells. The extend of metastasis was evaluated after 28 days.

Results: We provided evidence that heparin has an additional biological activity which inhibits binding of P- and L-selectin to their natural ligands. Since also modified heparins without any anticoagulant activity were found to attenuate efficiently metastasis, the heparin effect on cancer progression may not be principally due to inhibition of coagulation. In fact, we have demonstrated that the selectins play critical roles during the hematogenous phase of carcinoma metastasis in animal models. Natural selectin ligands on carcinoma cells were identified as mucins carrying sialylated, fucosylated and sulfated carbohydrate structures. Heparin treatment in wild type mice resulted in attenuation of metastasis similar to the one observed either P- and/or L-selectin in mice, indicating that inhibition of selectins significantly contributes to the anti-cancer effect. Inhibition of selectin-mediated interactions with carcinoma cells was found to be critical for early stages of a metastatic cascade. Most importantly, attenuation of metastasis can be achieved at clinically acceptable dosages. In addition, heparin contains also other biological activities including inhibition of heparin-induced modulation of cytokines and growth factors activities.

Conclusions: The available evidence from preclinical analyses, together with the promising observations from clinical trials, merits further investigation of heparin as a potential anti-metastatic therapy.

Serum and Alveolar Concentrations of Antibiotics during the Treatment of Ventilator-Associated Pneumonia

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Background: We assessed the serum and alveolar concentrations of antibiotics frequently used during the treatment of ventilator-associated pneumonia (VAP). We also analyzed critically ill patients in order to optimize antimicrobial treatment in this particular population.

Methods: Various antibiotics (β-lactams, tobramycin, levofloxacin or linezolid) were administered in intermittent or continuous infusion to critically ill patients with VAP. At steady-state (after two days of therapy), blood samples were withdrawn from each patient and serum concentrations were measured by high-performance liquid chromatography. Simultaneously to blood sampling, antibiotic concentrations were determined in epithelial lining fluid (ELF) obtained from standardized mini-bronchoalveolar lavages, which is a reliable method for the measurement of alveolar drug concentrations.

Results: The 122 patients were of similar age (50-70 yrs), weight (60-80 kg) and creatinine clearance (60-100 mL/min). The antibiotic daily dose for each patient, administered in intermittent or continuous infusion, the serum and ELF concentrations and the ELF:serum concentration ratios appear in Table 1.

Conclusions: Serum and alveolar concentrations and alveolar diffusion of antibiotics from different and similar classes exhibit wide variations in critically ill patients with VAP. Individual antibiotic dosages may be helpful to optimize the administration regimen of antibiotics and therefore PK/PD parameters (T1/2, Cmax/MIC and AUIC/MIC) during the treatment of VAP.

Table 1. Mean steady-state serum and ELF antibiotic concentrations

<table>
<thead>
<tr>
<th>Antibiotic (dose/day)</th>
<th>Serum concentration (mg/L)</th>
<th>ELF conc. (mg/L)</th>
<th>ELF:serum conc. ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intermittent</td>
<td>Continuous</td>
<td></td>
</tr>
<tr>
<td>Cefazolin 4 g</td>
<td>10</td>
<td>13.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Cefazolin 4 g</td>
<td>15</td>
<td>13.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Ertapenem 1 g</td>
<td>150</td>
<td>0.8</td>
<td>0.21</td>
</tr>
<tr>
<td>Levofloxacin 500 mg ep</td>
<td>12</td>
<td>3.0</td>
<td>0.25</td>
</tr>
<tr>
<td>Levofloxacin 500 mg ep</td>
<td>12</td>
<td>3.0</td>
<td>0.25</td>
</tr>
<tr>
<td>Linezolid 600 mg bid</td>
<td>16</td>
<td>17.6</td>
<td>1.12</td>
</tr>
<tr>
<td>Linezolid 600 mg bid</td>
<td>16</td>
<td>17.6</td>
<td>1.12</td>
</tr>
<tr>
<td>Piperacillin/tazobact 125 mg</td>
<td>10</td>
<td>-</td>
<td>0.45</td>
</tr>
<tr>
<td>Piperacillin/tazobact</td>
<td>10</td>
<td>-</td>
<td>0.45</td>
</tr>
<tr>
<td>Tobramycin 1-6 mg/kg</td>
<td>10</td>
<td>38.9</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Therapeutic effects of Crocus sativus (saffron) on respiratory diseases

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As indicated in ancient Iranian medical books Crocus sativus (Iridaceae) or saffron has therapeutic effects on respiratory diseases. Therefore in a series of experiments, the following effects of cumulative concentrations of saffron and its constituent, saffaran were examined on tracheal chains of guinea pig: relaxant, stimulatory effect at β-adrenoceptors, inhibitory effect at histamine (H1) receptors and the inhibitory effects on muscarinic receptors in precontracted trachea by 10 μM methacholine (muscle). In the relaxant effect, extract and saffaran showed significant relaxant effects compared to that of saline (p<0.05 to p<0.001). In precontracted trachea by 60 μM KCL (group 2) and also histamine, extract and saffaran showed concentration dependent relaxant effects compared to that of saline (p<0.05 to p<0.001 for different concentrations except two low concentrations of saffaran). However, in group 3 (precontracted incubated tissue with both concentrations of the extract and saffaran) the extracts of Crocus sativus showed a weak relaxant effect (p>0.05 only for highest concentration).

There were clear leftward shifts in isoprenaline curves obtained in the presence of only higher concentration of the extract in group 2 (incubated tissues with saffaran) and its concentration significantly higher than that of saline (p<0.005 to p<0.001). The maximum responses obtained in the presence of all concentrations of the extract and saffaran in group 2 were significantly lower compared to saline (p<0.05 to p<0.001). The maximum responses obtained in the presence of all concentrations of the extract and saffaran in group 2 were significantly lower than that of saline (p<0.05 to p<0.001) respectively. The maximum responses obtained in the presence of all concentrations of the extract and saffaran in group 2 were greater than those of group 1 and 3 (p<0.05 to p<0.001). The maximum responses obtained in the presence of all concentrations of the extract and saffaran in group 2 were significantly lower than that of saline (p<0.05 to p<0.001). The maximum responses obtained in the presence of all concentrations of the extract and saffaran in group 2 were significantly lower than that of saline (p<0.05 to p<0.001). The maximum responses obtained in the presence of all concentrations of the extract and saffaran in group 2 were significantly lower than that of saline (p<0.05 to p<0.001).

The results of inhibitory effects on muscarinic receptors showed clear parallel rightward shifts in methacholine-response curves obtained in the presence of atropine. Low concentrations of saffaran and extract compared with the curves obtained in the presence of saline. The EC50 (the effective concentration of saffaran, causing 50% of maximum response) obtained in the presence of all concentrations of the extract and saffaran in group 1 and only in the presence of the two concentrations of the extract in group 2 was significantly lower compared to saline (p<0.05 to p<0.001). The maximum responses obtained in the presence of all concentrations of the extract and saffaran in group 2 were significantly lower than that of saline (p<0.05 to p<0.001).

The results showed a potent relaxant effect of saffron, a relatively potent stimulatory effect of the extract from Crocus sativus on β-adrenoceptors, an inhibitory effect at histamine H1 receptors and a possible inhibitory effect at muscarinic receptors in the trachea of guinea pigs. The results also indicated that the saffaran is at least in part, responsible for the relaxant effect of Crocus sativus.
Different therapeutic effects of Nigella sativa

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In a series of studies the following effects of Nigella sativa on guinea pig tracheal chains and human airways were studied. The results showed: (1) Aqueous and macerated extracts from Nigella sativa showed significant relaxant effect compared to saline (p<0.001). The extracts caused the non parallel rightward shift in methacholine concentration response curve and EC50 methacholine obtained in the presence of extracts were significantly greater than that of saline (p<0.05 to p<0.001). (2) Plant extracts caused parallel rightward shifts in histamine concentration response curves obtained compared to saline. The EC50 histamine in the presence of extracts were significantly greater than saline (p<0.05 to p<0.001). (3) There was a leftward shift in scopolamine concentration response curve in the presence of aqueous extract. (4) Different extracts showed significant relaxant effect on tracheal chains incubated with Chlorpheniramine and propranolol, contracted by methacholine (p<0.01 to p<0.001) but did not show any relaxant effect on tracheal contracted by KCl. (5) Aqueous extract caused a rightward shift in the CaCl2 response curves and EC50 CaCl2, the presence of extract was significantly greater than that of saline (p<0.05). (6) However, thymoquinone, main constituent of the plant did not show any significant relaxant effect on tracheal chains. (7) Boiled extract of this plant caused significant increases in all measured pulmonary function tests (PFTs) (p<0.05 to p<0.001) comparable to the effect of ephedrine. (8) Two months administration of boiled extract also caused significant improvement in PFT values, respiratory symptoms, chest wheezing and drug usage in asthma patients. (9) Concentrations of extracts of the plant showed significant reduction of cough severity (p<0.01 for all cases) which were significantly greater than that of codeine (p<0.05-P<0.001).

Results showed a potent relaxant effect of Nigella sativa on tracheal chains, a relatively potent bronchodilatory effect on asthmaic airways and a potent antitussive effect.

Neurotensin Agonists: Novel Analgesics with Synergy to Morphine

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Background: Neurotensin (NT) is a widely distributed neuropeptide in the central nervous system that modulates nociception at several different levels, but is associated with hypotension and hypotension. NT exerts its effect through NT receptors, of which there are three known subtypes (NTS1, NTS2 and NTS3). Morphine is a μ-opioid receptor agonist that is commonly used for the treatment of many pain conditions. It is usually associated with side effects that can be serious. We hypothesize that selective NT receptor agonists may represent a novel class of analgesics and their use in conjunction with morphine will have synergistic properties with morphine which may reduce the dose administered and its side effects.

Methods: Studies were done to test the use of a new peptide analogue of NT (NT69L) as a new class of analgesics, acting through the NT receptors, alone and in combination with morphine. The antinociceptive activity of NT69L and morphine was studied in rats using the hot plate test to determine if there is synergism between the two drugs in reducing pain. The NTS2 receptor antagonist, levcocabastine was used to determine the receptor subtype involved in the synergistic effect of NT69L and morphine.

Results: The administration of both NT69L and morphine resulted in a dose-dependent analgesic effect. Isotonic analysis was used to study the antinociceptive interactions between the two drugs. The isotonic analysis demonstrated that the combination of sub-analgesic doses of NT69L and morphine was synergistic in the hot plate test. Pretreatment with the NTS2 receptor antagonist, levcocabastine, attenuated the synergistic effect of NT69L and morphine on the hot plate test.

Conclusion: The results provide preliminary data supporting the hypothesis that the synergistic combination of NT69L and morphine would improve the pharmacological treatment of pain while minimizing specific adverse effects of each of the drugs at a higher dose. Both NT receptors NTS1 and NTS2 are important for the synergistic effect of NT69L and morphine.

Current Medical Countermeasures (Vaccines-Antibodies- Antibiotics) to Protect Humans from the Anthrax Bioterrorism

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Background: B. anthracis spore attacks through the US mail system have demonstrated their feasibility as a bioterrorism weapon. Vaccination appears to be the most effective and economical form of mass protection, however, the current vaccines have drawbacks that justify the immense efforts for the development of improved treatment modalities. This review summarizes the current human clinical studies of postattack prophylactic vaccination with anthrax toxins in the current humans approaches developed mainly since the 2001 events against inhalational anthrax.

Methods – Results: The increased research activity has led to a huge expansion in the existing literature. The present work is based on an extensive review of the international literature. Combination of postattack prophylactic vaccination with antibiotic therapy is the most effective strategy. Current approaches against anthrax toxins are focused on agents that affect crucial steps of the intoxication process. High-affinity toxin-specific monoclonal antibodies have a significant clinical effect, if they are administered rapidly. While effective antibiotics, anthrax toxins and vaccines are available, concerns over their safety and effectiveness have driven the development of 2nd and 3rd generation products that act rapidly and with minimal adverse effects. Protective antigen (PA) is the principal immunogen of the 1st generation licensed vaccines. A 2nd generation vaccine is based on highly purified recombinant PA (rPA) and is likely to receive licensing approval. The 3rd generation vaccines aim to enhance the efficacy of the previous vaccines. They would ideally be given via the oral, nasal or dermal routes for delivery of rPA in a single dose facilitating stockpiling and mass vaccination programs. DNA vaccination could form the basis for multigen vaccine development. The development of novel agents is hampered by the difficulty in demonstrating effectiveness in humans.

Conclusions: Treatment response to a deliberate release of B. anthracis spores includes the prompt administration of antibiotics. There are concerns for the availability of antibiotics and vaccines over their effectiveness and toxicity. Despite the intensive anthrax research, there has been as yet no real progress. The huge efforts are expected to provide an array of novel protective agents and their tailoring list reflects the emergency of the global community to combat the anthrax threat.
Cefepime Neurotoxicity in Perspective

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Abstract: Cefepime, a cephalosporin, is often used in the treatment of infections, particularly in the intensive care unit. However, the drug has been shown to be neurotoxic, particularly in patients with renal failure. This study aimed to investigate the potential neurotoxic effects of cefepime in vitro and in vivo.

Methods: The study was divided into two phases: in vitro and in vivo. In the in vitro phase, the neurotoxic effects of cefepime on cortical neurons were assessed using a cell culture model. In the in vivo phase, the drug was administered to rats and its effects on the brain were monitored.

Results: The in vitro study showed that cefepime had a significant neurotoxic effect, as evidenced by the increased release of neurotransmitters. The in vivo study showed that the drug led to a decrease in the activity of the hippocampus, as measured by the reduced number of dendritic spines.

Conclusions: Cefepime is a potent neurotoxic agent, particularly in patients with renal failure. Therefore, it is important to monitor patients for neurotoxic effects and to consider alternative treatment options in these patients.

Keywords: Cefepime, Neurotoxicity, In Vivo, In Vitro.
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Cardiovascular disease represents a major clinical problem affecting a significant proportion of the world's population and it remains the major cause of death in the EU and the rest of the Western world. Furthermore, the burden on healthcare systems is increasingly high; the overall cost of cardiovascular disease to the EU economy is estimated to be in excess of 192 billion Euros per year. The majority of therapies currently available for the treatment of cardiovascular disease do not cure the problem but merely treat the symptoms. Furthermore, many cardiovascular drugs have serious side effects and have narrow therapeutic windows that can limit their usefulness in the clinic. Thus, the development of more selective and highly effective therapeutic strategies that could cure specific cardiovascular diseases would be of enormous benefit both to the patient and to those countries where health care systems are responsible for an increasing number of patients. There is increasing evidence to suggest that targeting the cell cycle machinery in cardiovascular cells (e.g. cardiac myocytes, vascular smooth muscle cells (VSMCs), endothelial cells) provides a novel approach for the treatment of certain cardiovascular diseases, including post-infarct heart failure, restenosis, in-stent stenosis and bypass graft failure. It has been demonstrated that certain cell cycle molecules that are important for regulating terminal differentiation in cardiac myocytes (e.g. cyclins, cyclin-dependent kinases [CDKs], CDK inhibitors, E2F transcription factors) can be targeted to reinitiate cell division and repair in the myocardium post-infarction. Furthermore, cell cycle molecules that control excessive VSMC proliferation in disorders such as restenosis, in-stent stenosis and bypass have also been targeted effectively in recent laboratory and clinical studies. The results of these studies illustrate the exciting possibility of targeting components of the cell cycle machinery to develop magic bullets to improve cardiac function and prognosis for patients with heart failure and for patients with atherosclerosis.

Epinephrine Vasoconstrictor Drug-Drug Interactions Revisited
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Drug-Drug interactions with regard to epinephrine vasoconstrictor local anesthetic formulations have been vastly overstated in the past. Such purported drug interactions include tricyclic antidepressants, non-specific beta blockers and cocaine. These supposed but mistaken interactions are widely published in many Dental Pharmacology Texts and have established problematic clinical considerations negatively influencing pharmacologic patient therapy. A portion of the rationale for such purported drug interactions include poorly designed studies and inapplicable case reports. The major misconceptions are due to misunderstandings of adrenergic pharmacology. Lack of understanding with regard to epinephrine’s beta two receptor’s influence upon blood pressure dynamics and the difference between alpha adrenergic local versus system effects are problematic. A misunderstanding of the positive attributes of local anesthetic vasoconstrictor action and limited knowledge of sympathetic activation and the actions of endogenous norepinephrine among dental clinicians has contributed to this problem and resulted in the misuse of pharamcotherapeutics. The lack of toxicity is further influenced due to epinephrine’s exceedingly short half-life. To add to the above, the mechanism of tricyclic antidepressant pharmacology is not common knowledge among dental clinicians and even such simple drug-drug interactions as the additive drug-drug interaction between cocaine and other local anesthetics is overlooked. Furthermore, many recent human studies which demonstrate the relative safety of epinephrine vasoconstriction will also be discussed. In conclusion, an evaluation of potential drug-drug epinephrine vasoconstrictor interactions is important with regard to clinical dental care and appears to limit fears regarding several noted but highly suspect previously accepted drug-drug interactions.

Magic bullets for the treatment of inflammatory bowel disease – yet to come?
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Background: Inflammatory bowel disease (IBD), an immune-mediated chronic intestinal condition, encompasses two idiopathic inflammatory diseases of the intestinal tract: Crohn’s disease and ulcerative colitis. Currently, medical treatment for IBD aims at induction and maintenance of remission. Established therapies, such as 5-aminosalicylic acid compounds, corticosteroids, immunomodulators or calcineurin inhibitors, however, lack specificity or effectiveness and might cause significant long term side effects. Recent advances in the knowledge of pathogenesis and immunology of IBD has led to the development of novel therapies directly targeting specific aspects of the inflammatory process, such as cytokines and receptors involved in T-cell activation, selective adhesion molecule blockers, anti-inflammatory cytokines, modulators of the intestinal flora or monoclonal antibodies. The first monoclonal TNFα antibody, has been successfully introduced into clinical practice for the treatment of IBD approximately 10 years ago. Most other novel therapies are undergoing different stages of clinical evaluation. Conclusions: Although during the last years progress has been made in both defining the mechanisms underlying the development of inflammatory bowel disease and expanding the spectrum of effective therapies, it might be too early to know, whether the currently tested compounds will be routinely employed in IBD treatment. It seems rather unlikely, however, that one single drug will prove to be a magic bullet.
**Vilnius, Lithuania LT-03101;**

**Conclusion:** substrates and efficiency of the inhibitors were evaluated.

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**All abstracts are listed in alphabetical order of the presenting author.**

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**Authors’ disclosure statement:** Imatinib and Nilotinib were provided by Novartis. The work was supported by Leukaemia E. V.

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**Background:** Nilotinib is a selective bcr-abl tyrosine kinase inhibitor that is 30-fold more potent than Imatinib in vitro. To examine the molecular and functional effects of Nilotinib and Imatinib we performed gene expression and functional analyses in K562 cells following in vitro treatment with the two tyrosine kinase inhibitors. Particular emphasis was put on 1539 genes which we found to be differentially expressed in primary CD34+ cells from patients with CML in chronic phase in comparison to CD34+ cells from normal bone marrow (Diez-Blanco et al., Leukemia 2006).

**Methods:** Affymetrix U133A+2.0 microarrays covering 21,722 probe sets were used to analyse the gene expression profile of 5x10^5 K562 cells after 24h in vitro treatment with Imatinib (0.05 µM) or Nilotinib (0.05 µM) (half maximal inhibitory concentration). FISH analysis confirmed the K562 cell line to be BCR-ABL positive. Gene expression data of the treated cells were compared with the data of untreated cells. In addition, proliferation (MTS Assay, Promega), apoptosis (Cell Death Detection ELISAPLUS, Roche) and cell cycle (FITC/BDuPkit, BD Pharmingen) assays were performed.

**Results:** Looking at those 1539 differentially expressed genes in K562 cells which distinguished patients with CML from healthy donors, we found that Imatinib led to a significant downregulation of 187 and upregulation of 45 genes. In general, the effect of Nilotinib with regard to the number of genes affected and degree of suppression was more pronounced resulting in the downregulation of 418 and upregulation of 41 genes. Of note, genes affected by Nilotinib included all genes altered by Imatinib such as those related to bcr-abl signaling. Downregulation of genes involved in cell cycle was only observed following Nilotinib exposure. The stronger effect of Nilotinib is in line with the results of cell cycle experiments showing that Nilotinib exposed cells had the lowest proportion of actively cycling cells. The proportion of apoptotic K562 cells was 5.5 fold greater following Nilotinib compared to Imatinib in apoptosis assays performed.

**Conclusion:** Nilotinib is apparently more potent than Imatinib with regard to the number of genes downregulated and the degree of their suppression. Many of the suppressed genes are associated with bcr-abl signalling and cell cycle.

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**Background:** Multidrug resistance (MDR) pumps, decreasing intracellular concentrations of antibiotics are major causes of resistance in opportunistic pathogens such as Pseudomonas aeruginosa. This phenomenon ensures the viability of bacteria under high concentrations of antibiotics and complicates the treatment of infected patients. Fast methods are needed to determine the efficiency of drugs inactivating MDR pumps for the successful treatment of patients.

**Methods:** Aerated suspensions of Escherichia coli and P. aeruginosa cells were studied in thermostated reaction vessels, and changes in the extracellular concentration of indicator lipophilic cation tetrathylphosphonium (TTP+) were measured using selective electrodes. Tetracycline was used as the model antibiotic, and, phenylalkyl arginyl β-naphthylamides (PAPN), reserpine and chlorpromazine were used as inhibitors of the different MDR pumps.

**Results:** Depending on the outer membrane permeability, membrane voltage and activity of the MDR pumps, cells and the main MDR pump mutants of P. aeruginosa and E. coli accumulated different amounts of TTP+. Addition of tetracycline and the pump inhibitors caused detectable alterations of the amount of TTP+ accumulating. Using the outer membrane-permeabilizing and the plasma membrane depolarizing compounds, activity of the pumps, affinity of the substrates and the efficiency of the inhibitors were measured.

**Conclusion:** Online monitoring of TTP+ fluxes across the bacterial envelope can be applied for studies of the activity of MDR pumps in E. coli and P. aeruginosa, for determination of the pump selectivity to substrates and for evaluation of the efficiency of pump inhibitors.

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**Online method for measuring of the activities of antibiotic efflux pumps in Escherichia coli and Pseudomonas aeruginosa**

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**Background:** Multidrug resistance (MDR) pumps, decreasing intracellular concentrations of antibiotics are major causes of resistance in opportunistic pathogens such as Pseudomonas aeruginosa. This phenomenon ensures the viability of bacteria under high concentrations of antibiotics and complicates the treatment of infected patients. Fast methods are needed to determine the efficiency of drugs inactivating MDR pumps for the successful treatment of patients.

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**Results:** Depending on the outer membrane permeability, membrane voltage and activity of the MDR pumps, cells and the main MDR pump mutants of P. aeruginosa and E. coli accumulated different amounts of TTP+. Addition of tetracycline and the pump inhibitors caused detectable alterations of the amount of TTP+ accumulating. Using the outer membrane-permeabilizing and the plasma membrane depolarizing compounds, activity of the pumps, affinity of the substrates and the efficiency of the inhibitors were measured.

**Conclusion:** Online monitoring of TTP+ fluxes across the bacterial envelope can be applied for studies of the activity of MDR pumps in E. coli and P. aeruginosa, for determination of the pump selectivity to substrates and for evaluation of the efficiency of pump inhibitors.
Abstracts

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Background: Well-known paradoxical effect that both high-dose estrogens and anti-estrogens cause tumor regressions. In this case important is an understanding of the properties of a tumor. Aims: 1) To study the influence of the same hormonal environment on tumor growth and character of change of the receptors level in tumors with different hormone sensitivity.

Methods: Concentration femtomoles/mg protein (fM/mg) of estrogen receptor (ER) was determined in transplanted mice mammary tumors with intensive reproduction mode (2-6 parturition without lactating) (n=20) and virgin receptor (ER) was determined in transplanted mice mammary tumors with the same hormonal environment on tumor growth and character of change of the receptors level in tumors with different hormone sensitivity. The levels of ER were determined by means of the dextran-coated charcoal technique. Mice of line C3H(Sin (n=69) contained in cages on 5 female +2 male (normal reproduction) in a room with daylight. Virgin female mice (n=47).

Results: The data provides both estrogen-positive and –negative tumors that actually are evoked from the same tissue, so that one can determine at what level(s) the hormone sensitivity or dependency exists. The widely accepted hypothesis that the interaction of estrogen with its cellular receptors determines the hormone dependency of mammary tumors should now be challenged on the basis of the following observations: a) tumors occurring in experimental animals with permanent high estrogen levels are receptor-negative (~20-100fM/mg) and with low estrogen levels (<2fM/mg) are receptor-negative; b) tumors regrowing after complete or partial regression as a result of endocrine ablation or hormone administration are no considered to be autonomous or hormone dependence but environment dependence; c) growth of a tumor at cyclically changing hormonal level leads to heterogeneity of mammary tumors that complicates hormone therapy.

Conclusions: 1) Series of parameters of a tumor such as the invasiveness, heterogeneity and others are consequence of adaptic properties of tumoral cells. 2) Ability of tumoral cells to adapt for change of a surrounding microenvironment answers on a question on a paradoxically of a hormone therapy. 3) High sensitivity of tumors to change of steroids concentration (10-8 M) discovers an epigenetic path of redifferentiation a tumor oncogenome i.e. multiple-stage therapy.

Pro-Atherogenic and Pro-Inflammatory Alterations in Mononuclear Cell Populations Induced by Oxidized Low Density Lipoprotein (oxLDL) and High Glucose Levels in Type II Diabetic Patients. Anti-Inflammatory Drugs, an Alternative?

BUSTAMANTE, M1, DÍAZ, F2, MUÑOZ, M4, GÜZMÁN, C1, LLANCAQUEO, A1, NÚÑEZ, L1, CAMPOS, L1, RIVAS, C1, VERA, J1, GROSS, H-C3 & BACHEM, M3.

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Background: Type II diabetes mellitus is a risk factor in the development of atherosclerosis. Two factors are central to the effect of diabetes, oxidative modification of LDL and high glucose concentrations. We hypothesized that mononuclear cells from diabetic patients in the presence of oxLDL and high glucose levels undergo pro-inflammatory alterations, which would enhance the development of atherosclerotic plaques. Therefore, our aims were: 1) To measure the induction of necrosis and apoptosis in mononuclear cells from diabetic patients, 2) To measure the release of pro-inflammatory interleukins in stimulated cells, and 3) To analyze the possible use of meloxicam as an alternative treatment.

Methods: This study included cells from 140 diabetic patients and 105 controls. The individuals were grouped by age, sex and obesity for the measurement of necrosis and apoptosis; 15 diabetic and 15 controls for quantification of interleukins; and 20 patients and 20 controls to study the effect of meloxicam (7.5 mg/day/30days). The results are presented as the mean ± SD of at least three independent experiments (a value of p < 0.05 was considered as significant, t-test).

Results: Cell necrosis and apoptosis was increased in mononuclear cells from obese, diabetic patients older than 50, without significant sex-related differences. Moreover, the release of pro-inflammatory interleukins, in particular those related with the development of atherosclerosis, was also increased in diabetic cells. Interestingly, meloxicam treatment decreased necrosis, apoptosis and release of pro-inflammatory interleukins from diabetic mononuclear cells.

Conclusions: Some of the pro-inflammatory effects observed when diabetic cells are subjected to the synergistic action of oxLDL and high glucose concentrations, such as necrosis, apoptosis and interleukin release, are reduced by treatment with meloxicam. Acknowledgment: FONDECYT 1040977, DIP-UCSC, Prof. Max Bachem.

Tacrolimus: a highly effective new therapy for chronic glomerular diseases?

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Abstract:

Tacrolimus is a potent immunosuppressive agent that has been demonstrated to be superior to cyclosporine after renal transplantation as an effective and safe anti-rejection drug. Based on the excellent outcomes in the transplant population, the use of tacrolimus has been extended to patients with a variety of different chronic glomerular diseases that are immune-mediated, such as steroid resistant nephropathies and lupus nephritis, to name a few. The aim of this presentation is to critically appraise data related to the safety and efficacy of tacrolimus in adults and children with immune mediated renal diseases, outside the transplant setting. New evidence discussing the potential benefits and side-effects of long-term tacrolimus use (such as subclinical nephrotoxicity) will be discussed, so as to allow the audience to make an evidence-based assessment on whether tacrolimus is a reasonable strategy to treat patients with chronic glomerulonephritis.
The two main problems in evaluating resistance to antiparasitic drugs in populations of naturally infected hosts: efficacy variability and cut-off value for resistance

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Background: Variability of parasite/host response to antiparasitic drugs is large in field conditions depending on drug and galenic formulation. Resistance to anthelmintics has been well documented in sheep and goats, partly in cattle and less frequently in man. We dispose of different evaluations of efficacy for anthelmintics, none of them being a golden standard. We do not really know up to what level of efficacy we consider that we are facing a resistance phenomenon. In that respect it has been considered that less than 90% (horses) or 95% (ruminants) efficacy was the limit for stating on resistance. We are in great need of evaluation of efficacy of drugs and cut-off value for resistance.

Methods: Since distribution of efficacy values is not known and is clearly not a Gaussian distribution, we propose a bootstrap confidence evaluation, using a freeware we constructed (Bootsstreat available on demand or on internet). The bootstrap evaluation is based on different evaluation formulas for efficacy (before/after treatment with and without untreated control, after treatment in treated and controls, either using arithmetic or geometric means). The evaluation of cut-off for resistance is a completely open problem. We propose a two steps’ method: transformation of individual efficacy data that results in a Gaussian distribution and then when efficacy is not real we suppose that Gaussian distribution is acceptable and progressively with increasing doses and for efficacy, there is a departure from such a distribution. We use data from anthelmintic treatments (tetramisole, macrocyclic lactones, benzimidazoles) in cattle or sheep.

Results: Bootstrap results indicate that usual procedure for estimating efficacy are not accurate since confidence intervals reach sometimes more than 20% of the mean. The cut-off values are highly dependent on drug/dose (example in tetramisole) and galenic form used (injectable or per os ivermectin). We propose a strategy for evaluating resistance in the field.

H pylori: Treatment for the patient only or the whole family?

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Aim: To compare the effects of treatment of H pylori-infected individuals with the effects of treatment of individuals as well as all H pylori-infected family members.

Methods: H pylori-positive patients with similar demographic specifications were prospectively randomized with respect to treatment, with a triple regimen of either antibiotics and all H pylori-positive family members living together (group I) or patients only (group II). Nine months after treatment, all patients were assessed for H pylori positivity.

Results: There were 70 H pylori-positive patients in each group; patients in groups I and II lived with 175 and 190 H pylori-positive relatives, respectively. Age, sex and H pylori positivity rate were similar in both groups of relatives. Nine months after 14 d standard triple therapy, H pylori positivity was 7.1% in group I patients and 38.6% in group II patients [P < 0.01, OR = 8.61 95% confidence interval (CI): 2.91-22.84].

Conclusion: The present results indicate bad environmental hygienic conditions and close intra-familial relationships are important in H pylori contamination. These findings indicate all family members of H pylori-positive individuals should be assessed for H pylori positivity, particularly in developing countries where H pylori prevalence is high; they also suggest patients, their spouses and all H pylori-positive family members of H pylori-positive individuals should be treated for H pylori infection.

Anticancer Activity and Lack of Toxicity of CZ48 Administered Orally To Nude Mice Carrying Xenografts of 22 Human Tumors

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Background: Camptothecin, an alkaloid extracted from the tree Camptotheca acuminata and some of its derivatives were found to have potent anticancer activity against human tumors growing as xenografts in nude mice. However, these compounds were much less active against tumors of cancer patients. Further studies established that all the camptothecins were converted to their inactive form in the human blood by the opening of their lactone ring caused by the presence of human serum albumin.

Methods and Results: In order to prevent such an opening, our laboratory has developed CZ48 (Camptothecin-20(S)-propionate hydrate). We have tested it for anticancer activity administering it orally to nude mice carrying xenotransplants of 22 human cancers (4 colon, 2 lung, 5 pancreas, 5 breast, 2 melanomas, and 2 sarcomas, 1 prostate and 1 bladder) at doses of 50 mg/kg/day to 2000 mg/kg/day daily, 5 to 7 days/week. The tumors were growing subcutaneously on the back of 3 month old nude mice. The treatment started when tumors were measurable by caliper (200-300 mm^3). CZ48 was administered suspended in cottonseed oil.

Conclusion: Two conclusions clearly emerge from this study: 1) CZ48 is totally devoid of toxicity at the maximum doses used which were administered for more than 300 days; and 2) CZ48 is a potent anticancer drug against human cancer xenografts with a wide spectrum of action which caused complete growth inhibition in 20 out of 22 human cancers treated.
Molecular strategies to improve the anti-tumour activity of Zoledronic acid in prostate cancer cells

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* Corresponding author

Background: Zoledronic acid (ZOL) is an amino-bisphosphonate able to inhibit the prenylation of intracellular proteins through the inhibition of farnesyl/geranylgeranyl synthase. Preylation is essential for the maintenance of the activation of components of signal transduction pathways regulating apoptosis and proliferation such as ras and raf-related proteins. ZOL has demonstrated a direct anti-tumour effect in vitro and in preclinical models and its ability in preventing skeletal related events is proven in patients with bone metastases from different origins. Clinical evidence on its direct anti-proliferative effects is emerging but its activity is limited by the excessive accumulation in the bone.

Conclusions: We describe several strategies in order to improve the anti-tumour activity of ZOL based on preclinical and biological rationale.

Methods: The first strategy is to combine ZOL with either cytostatic drugs or other biologic agents such as the farnesyl transferase inhibitors. The second strategy is to find new molecular targets of ZOL through the use of technological platforms such as microarray data.

Results: We have analysed the gene modulation induced by ZOL in androgen-resistant prostate cancer PC3 cells with cDNA microarray platform to identify new molecular targets of ZOL in prostate cancer. The gene coding for cytokine-rich, angiogenic inducer, 61 (CYR61), often overexpressed in tumour cells, resulted highly down-regulated with a fold-change of 5.68. Furthermore, we have studied the effects of different concentrations of ZOL on CYR61 protein and we have found that CYR61 protein expression was significantly decreased after exposure to ZOL on both PC3 and DU145 cell lines. The effect of ZOL on CYR61 expression was dose and time dependent and was due to a reduced transcriptional activity of CYR61 promoter as demonstrated by transfection with a plasmid encoding for luc-CYR61 promoter. Interestingly, other signal transduction inhibition inhibitors did not induce or induced less effect on CYR61 modulation if compared to ZOL. Moreover, ZOL reduced CYR61 expression through decreased activation of ras/raf-1 dependent pathway that was dependent from epidermal growth factor or tumor necrosis factor-α stimulation. Finally, we have investigated the role of CYR61 in the regulation of growth inhibition and invasion/motility of PC3 cells using a shRNA for CYR61 in order to downregulate the expression of CYR61 protein. We have found that shCyR61 enhanced ZOL inhibition of proliferation induced by ZOL. Since CYR61 was reported to be involved in the resistance to therapies we have evaluated if ZOL could sensitize PC3 cells to Docetaxel (DTX). We have found a sequence-dependent synergism induced by the combination between ZOL and DTX on PC3 cell growth inhibition and similar results were recorded after treatment of PC3 cells with shCyR61.

Conclusions: In conclusion, it is possible to design new molecular-rational-based therapeutic strategies in androgen-independent prostate cancer based on CYR61 modulation.

Vascular disruption: an old mechanism rediscovered for targeting tumor blood supply

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Background: Vascular disrupting agents (VDAs) are cancer drugs that act through destruction of tumor neovascularization. The majority of known VDAs act through tubulin binding which leads to destabilization of microtubules in neovascular endothelial cells, increased vascular permeability, loss of perfusion and tumor necrosis. Many VDAs are currently in all phases of clinical development. The preclinical and clinical history of MPC-6287, a quinazoline-based tubulin targeting agent under development by Myriad Pharmaceuticals, is presented as a case study for discovery and development of a VDA.

Methods: MPC-6287 was identified through a drug discovery process that began with a screen for apoptosis induction in a cancer cell line and continued through biochemical, cellular and animal model characterization of activity. Subsequently, safety and efficacy of MPC-6287 was studied in two Phase I clinical trials.

Results: MPC-6287 was found to compete with colchicine for tubulin binding and to destabilize tubulin assembly using biochemical and cellular assays. MPC-6287 was also observed to potently kill tumor and endothelial cells in culture and rapidly damage tumor neovascularization, induce tumor necrosis and inhibit tumor growth in xenograft models. Furthermore, MPC-6287 did not show evidence for the capacity to cause cell toxicity in tumor cells overexpressing the ABC transporters, P-gp (MRP-1), BCRP-1 which mediate multidrug resistance and other blood-brain barrier.

MPC-6287 demonstrated high brain availability in mice, with exposure in excess of 14-times that of plasma. A maximum-tolerated dose (MTD) of 3.3 mg/m2 was elucidated in a Phase I clinical trial.

Conclusions: MPC-6287 is a potent cytotoxic agent that inhibits tumor growth 100-fold through vascular disruption. MPC-6287 is non-toxic through vascular disruption. MPC-6287 is non-toxic through vascular disruption.

The dynamic hypothesis of latent tuberculosis infection offers a new rational to develop future therapeutic strategies.

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Background: It has been postulated that once infected by Mycobacterium tuberculosis bacilli can remain in latent lesions. With the aid of resuscitation factors these bacilli can reactivate and induce disease. Considering the constant cellular turnover and healing of injured tissues; How can a 9-month treatment with isoniazid eliminate the dormant bacilli, while immersed in old lesions?

Conclusions: 1) B-cell epitope prediction is potentially complicated by the presence of cysteine and histidine. 2) The evaluation of B-cell epitope predictions against empirical data is meaningful only if the two pertain to exactly the same types of antibody (antipeptide or antiprotein) and antigen (peptide or protein). 3) Predictions on genuine antibody-antigen cross-reactivity can be evaluated against empirical data in the case of interaction between antipeptide antibody and protein antigen.

Results: Structural-energetic parameters could not be unambiguously assigned to cysteine in view of its capacity for disulfide bond formation. The energetic contribution of histidine could not be determined in view of the uncertainty of its protonation state at physiologic pH. The binding contexts defined by the types of participating antibodies (antipeptide or antiprotein) and antigens (peptide or protein) were all fundamentally different from one another. Predictions on genuine antibody-antigen cross-reactivity could be evaluated against empirical data only with regard to interaction between antipeptide antibody and protein antigen.

Methods: Structural-energetic analysis was applied to peptide and protein antigens. A possible rate-limiting process of local epitope unfolding was considered for the cross-reaction of antipeptide antibody with protein antigen. Immunodominance was treated as a thermodynamically determined hierarchical steric-exclusion phenomenon. The algorithm thus developed was implemented as a computer program SAPPHIRE (Structural-energetic Analysis Program for Predicting Humoral Immune Response Epitopes), with the estimated affinity for antibody as the main criterion for epitope prediction. Predictions were rendered on the cross-reactivities of polyclonal antibodies to 38 peptides with 15 globular proteins of known structure and evaluated against published experimental data comprising 18 positive and 20 negative binding interactions.

Background: B-cell epitope prediction facilitates the design of antibody-binding constructs for the development of novel vaccines and immunodiagnostics. This work aimed to gain insights into the problem of B-cell epitope prediction using structural energetics.

Conclusion: It has been postulated that once infected by Mycobacterium tuberculosis bacilli can remain in latent lesions. With the aid of resuscitation factors these bacilli can reactivate and induce disease. Considering the constant cellular turnover and healing of injured tissues; How can a 9-month treatment with isoniazid eliminate the dormant bacilli, while immersed in old lesions?

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Comparison of oral pharmacokinetics of nifedipine in different populations

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Background: Nifedipine is a calcium channel blocker that is widely used in the treatment of hypertension and angina pecto ris. It has been described that this drug is importantly metabolized by CYP3A4 and differences in its pharmacokinetics have been described. In fact, it has been suggested that plasma levels are higher in Nigerians, South Asians and Mexicans when compared with Caucasians. In order to extend this information, the purpose of this study was to evaluate the oral pharmacokinetics of nifedipine in Mexicans and to compare the pharmacokinetic parameters reported in different populations by means of a meta-analysis.

Methods: Twenty male healthy volunteers were enrolled in this study that was approved by the Institutional Research and Ethics Committees. All were fit according to medical history, clinical examination and suitable laboratory tests. After an overnight fast, subjects received an oral dose of 10 mg nifedipine and blood samples were collected during 8 hours. Plasma was analysed by a validated HPLC method. Pharmacokinetic parameters were obtained by non-compartmental approach and compared by meta-analysis with those reported in different populations.

Results: Nifedipine was rapidly absorbed reaching the maximum between 30 to 60 minutes, then decreased with a half-life of about 5 hours. When pharmacokinetic parameters obtained in this study were compared with those reported in other populations by meta-analysis, it was observed that AUC reached in non-caucasian (Asian, African and Mexican) populations was almost twice the reported in Caucasians, indicating interethnic differences in the oral pharmacokinetics of this drug.

Conclusions: It is confirmed the existence of interethnic differences in the oral pharmacokinetics of nifedipine, non-caucasians reaching higher levels than Caucasians and therefore, blindly extrapolation of dosage regimens between populations is not adequate for this drug.

Electrochemical Behavior of Flavonoids in the Presence of Metal Ions

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Background: Flavonoids are a large group of phytochemicals ubiquitously found in many food products of vegetable origin. They have been reported to have a broad spectrum of pharmacological activity. In addition, due to the presence of hydroxy groups in their molecular structure, flavonoids exhibit strong antioxidant properties. Previous works in this field showed that their antioxidant activity is modified in the presence of metal ions. In this work we report the electrochemical behavior of flavonoids in the presence of the metal ions Cu (II), Fe (III) and Ag (I). Our results show that the interaction of these metal ions with flavonoids changes the redox behavior of the flavonoids, with the flavonoid-Cu(II) interaction likely to be the most significant, followed by the flavonoid-Fe(III) interaction. The present work is to make a comparative study on the electrochemical behavior (cyclic voltammetry) of the flavonoids quercetin (qrc), rutin (rut), rhamnetin (rha) and isorhamnetin (irh) in the presence of the metal ions Cu(II) and Fe(III).

Methods: Electrochemical behaviour of flavonoids in the presence of metal ions. The electrochemical cell was composed of a working gold electrode, an Ag/AgCl reference electrode and a platinum counter electrode. The solutions were prepared in a 1X10⁻³ M stock solution of flavonoids of the mentioned species, and adjusted to the desired pH with dilute NaOH or HClO₄. The potentials were scanned within the range 0.05 Vs SHE to 0.87 Vs SHE, at a scan rate of 0.005 Vs S⁻¹. The flavonoids quercetin (qrc), rutin (rut), rhamnetin (rha) and isorhamnetin (irh) 0.1X10⁻³ M, were employed.

Conclusions: The study of the electrochemical behavior of the flavonoids in aqueous solution is a powerful tool to understand the redox processes related to the Antioxidant Activity of these natural antioxidants. The oxidation of the catechol moiety producing a quinone can be split into two independent signals and individually analyzed. Further oxidation of the remaining OH groups of the quinone can also be studied in this medium, as well as the reduction of the flavonoids. The cyclic voltammetry of flavonoids in the presence of metal ions, changes the electrochemical behavior of the flavonoids, with the flavonoid-Cu(II) interaction likely to be the most significant, followed by the flavonoid-Fe(III) interaction.

Electrochemical Behavior of Flavonoids in the Presence of Metal Ions

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A vaccine made up by an algal β-glucan (laminarin, β-1,3-glucan with occasional β-1,6 single glucose side chains), conjugated with diphteria toxoid as a carrier protein component , protects against infections by different fungi and induces antibodies capable of inhibiting fungal growth. This is a sort of "cross-kingdom" vaccine because the immunizing antigen and the vaccination target belong to two different kingdoms, and this is certainly the first case in the field of human vaccines, which are generally based on the dogma "one or more specific antigens against one disease". Thus, it is "heretically" possible to convey in a single immunological tool the potential to protect against multiple infections, in our case all those caused by β-glucan −expressing fungi or bacteria. The generation of antibodies with the potential of directly inhibiting the growth of, or killing the fungal cells also opens an exciting perspective for both active and passive vaccination in immunocompromized subjects.

The above approach could be theoretically extended to non-fungal infections by selecting the appropriate molecular pattern shared by a given microbial group (e.g. peptidoglycan for Gram positive bacteria). Noteworthy, the molecular patterns are those microbial molecules which foster natural immunity through their binding to the pattern-recognition structures on host cells. Thus, single-component, molecular-pattern-based vaccines would merge the broad target range typical of innate immunity with the highly focused specificity of the adaptive immunity.
Results: The disease was confined to the right hepatic lobe in 76% of the patients and to the left lobe in 7%, in 17%, both lobes were affected. Fifty-eight patients (76%) had a single PLA and 18 patients had multiple abscesses. Etiology was biliary in 38%, hematogenous in 11%, posttraumatic in 9% and cryptogenic or attributable to rare reasons in the remaining patients. Microbiological culture was sterile in 24%, which was at least partly due to antibiotic pre-treatment. Staphylococcus, Streptococcus and E. coli were most often identified. Anaerobes were found in 15%. Factors associated with the need for surgery included empyema of the gallbladder, underlying malignancy, perforation, vascular complications (hepatic arterial thrombosis) and foreign bodies (e.g., toothpick, infected ventriculo-peritoneal shunt). In patients with biliary fistula it was important to ensure prompt bile flow (for instance, by sphincterotomy/stenting).

Conclusions: Additional therapy with interventional radiology and/or surgery was usually required in our patients and successful management of underlying diseases played a decisive role for positive outcome.

Methods: Clinical data of a series of 76 patients with PLA were analyzed. Initially, broad-spectrum antibiotics were given, and treatment was modified according to sensitivity testing as soon as possible. When indicated, additional therapy with percutaneous puncture/drainage, endoscopic papillostomy/stenting or surgical interventions was used.

Bioinformatic and Clinical Analysis of Prostate-Specific Antigen (PSA) Genes

Methods: In vitro and in vivo studies were carried out using prostate tumor (PC-3M) cells in culture and in nude mice. We analyzed modulation of protein expression in PC-3M cells by PSA using 2D-DIGE analysis coupled with HPLC-MS/MS and SEQUEST data mining. Biological network analysis was carried out using MetaCore integrated software designed for functional analysis of experimental data. Gene expression data for several regulated proteins were confirmed by real-time, quantitative PCR. Anti-angiogenic potential of PSA was also observed using human umbilical vein endothelial cells (HUVEC) in an in vitro anti-angiogenic assay.

Results: a. Incubation of PC-3M cells with purified PSA resulted in a significant down-regulation of expression of 147 genes including genes like VEGF, IL-8, EPHA2, CYP61, Bcl2, Pim-1 oncogene, and p53, that are associated with angiogenesis/tumor progression. b. Forty one genes were significantly (p<0.05) changed in abundance in PC-3M cells treated with PSA. Many down regulated proteins including Bcl2 protein and Vimentin, Dl-1 and HGF are known to be involved in tumor progression. c. PSA inhibited significantly, in a dose dependent manner, the migration/chemotaxis and attachment functions required for lube formation by HUVEC. d. PSA delivered to PC3M xenografts engrafted to nude mice resulted in inhibition of tumor growth.

Conclusions: 1. In PC-3M cells, PSA down regulates angiogenic factors and up regulates anti-angiogenic growth factors. 2. Enzymatically active and inactive forms of PSA have anti-angiogenic activity in vitro.

PSA has anti-tumor activity against PC-3M xenografts in nude mice.
Novel Clinically Relevant Proteasome Inhibitors and HDAC Inhibitors

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Background: Agents with specific biologic targets, that display more selective killing of leukemia cells as compared to normal lymphocytes require further study in these diseases. Here we focus on two distinct classes of new agents: proteasome inhibitors and histone deactyleas inhibitors (HDACi) and highlight a specific agent within each class as possessing unique properties with potential therapeutic benefit. NPI-0052 is a proteasome inhibitor distinct from bortezomib, which is approved by the Food and Drug Administration (FDA). PCI-24781 is an HDACi which targets a distinct class of HDACs more specifically than do vorozostat, the only FDA approved HDACi. Here we tested these compounds in leukemia cells to determine the mechanism of cytotoxicity and to compare them to approved counterparts.

Methods: Cell lines were representative of acute myelogenous leukemia and acute lymphocytic leukemia. Cell death was assessed by measuring DNA fragmentation by propidium iodide staining and flow cytometry. Caspase activation was measured by activity assays and by western blotting. Oxidative stress was quantified using dichlorofluorescein and dihydroethidium to measure levels of intracellular peroxides and superoxide, respectively. Proteasome activity assays were conducted using fluorogenic peptides. Histone acetylation was assessed by western blotting for histone H3 acetylation. Combination indices were based on Chou and Talalay’s methods.

Results: Dose response and time course studies revealed that NPI-0052 is more potent than bortezomib and inhibits the catalytic activity of proteasome more effectively than bortezomib. Similarly, PCI-24781 exerted unique effects, causing histone hyperacetylation at lower doses than vorozostat. Cells lacking caspase-8 did not display histone acetylation by PCI-24781. Surprisingly, NPI-0052 did not display histone acetylation which was dependent upon caspase-8 and oxidative stress. Synergy of NPI-0052 with several HDACi was stronger than seen with bortezomib. This agent also acetylates histones in an oxidant and caspase-8 dependent manner and synergizes with HDACi in killing of leukemia cells as compared to normal lymphocytes require further study.

Conclusions: 1) NPI-0052 is more potent alone and in combination with HDACi than bortezomib. This agent also acetylates histones in an oxidant and caspase-8 dependent manner or (2) PCI-24781 action was also dependent upon caspase-8, suggesting that promoting caspase-8 activity may complement the activity of both agents in leukemia.

Competing Causes of Death from a Randomized Trial of Extended Adjuvant Endocrine Therapy for Breast Cancer: NCIC CTG MA.17

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Background: Older women with early-stage breast cancer experience higher rates of non-breast cancer-related death. We examined factors associated with cause-specific death in a large cohort of breast cancer patients treated with extended adjuvant endocrine therapy. The aim of this study was to investigate the genotoxicity of nicotine and corresponding DNA damage in the mouse model.

Methods: In the MA.17 trial conducted by the National Cancer Institute of Canada Clinical Trials Group, 5170 breast cancer patients (median age = 62 years; range = 32–94 years) who were disease-free after approximately 5 years of adjuvant tamoxifen treatment were randomly assigned to treatment with letrozole (2583 women) or placebo (2587 women). The median follow-up was 3.9 years (range = 0–7.0 years). We investigated the association of 11 baseline factors on the competing risks of death from breast cancer, other malignancies, and other causes. All statistical tests were two-sided likelihood ratio criterion tests.

Results: During follow-up, 256 deaths were reported (102 from breast cancer, 50 from other malignancies, 100 from other causes, and four from an unknown cause). Non-breast cancer deaths accounted for 69% of the 252 known deaths (72% for those ≥70 years and 48% for those <70 years). Two baseline factors were differentially associated with type of death: cardiovascular disease was associated with a statistically significant increased risk of death from other causes (P = 0.02) and osteoporosis was associated with a statistically significant increased risk of death from other malignancies (P = 0.05). An increased risk of breast cancer-specific death was associated with lymph node involvement (P < 0.001) and increased risk of death from all three causes was associated with older age (P < 0.001).

Conclusions: Non-breast cancer-related deaths were more common than breast cancer-related deaths in this cohort of 5-year cancer survivors, especially among older women.

The Antagonistic Role of Curcumin against Nicotine induced Genotoxicity on different Organs of Female Rats under Restricted Dietary Protein

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Background: Nicotine, a major pharmacologically active substance of tobacco for several diseases, has proven to be a potential genotoxic compound. It is absorbed through lungs with smoking and mainly metabolized in liver, yet its effect on liver injuries and other organs particularly in restricted protein diet are not clear. The main aim of this study was to investigate the genotoxic effects of nicotine in liver, ovary and uterus of female populations particularly who used tobacco but deprived of healthy diet.

Methods: The study was investigated by: 1) Measurement of total DNA concentrations and 2) Analysis of DNA damage by Comet assay in the investigated tissues of female rats maintained under altered protein diets. Two groups of female albino rats (15 animals each) were maintained in normal protein diet (18% casein) and restricted protein diet (5% casein) respectively. Each group was divided in three subgroups experimentally (n=5). First subgroup was served as control and second and third subgroups (experimental) were injected nicotine tartrate (2.5 mg/kg body weight/day) subcutaneously and the third subgroup was administered curcumin (80 mg/kg body weight/day) orally. The animals were sacrificed after 21 days of treatment and the total DNA content in the specified tissues were measured. The DNA damages in the tissues were determined by Comet assay. Results were analyzed by One way Analysis of Variance, all pair wise Multiple Comparison Procedure (Holm-Sidak) and Function CORREL of Microsoft Excel.

Results: Total DNA contents of all investigated tissues were decreased more significantly (P<0.001) by nicotine in the dietary conditions. Significant (P<0.01) increase of total DNA content in normal diet and more significant (P<0.001) increase of that in protein restricted diet was observed due to curcumin supplements. Curcumin more significantly reduced the DNA damage percentage of the liver tissues in protein-restricted condition.

Conclusions: 1) The degree of nicotine-induced genotoxicity increases in protein restricted condition. 2) Curcumin effectively reduces the effect of nicotine as observed in tissues. 3) The protective role of curcumin is more pronounced under protein-restricted condition.
Synergistic inhibition of taxol-resistance primary ovarian cancer cells by oridonin and wogonin

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Abstract: In investigating the molecular principle of Chinese herbal medicine for cancer therapy, we use the composition of the original US phytod product "PC SPES" as a study model. Now no longer available, PC SPES was reported by several research groups to be active in suppressing hormone refractory prostate cancer cell growth. In the isolated, phytochemicals, baicalin, oridonin, and isoliquiritigenin and wogonin were found to individually or combinatorially inhibit the ovarian cancer cell lines sensitive (A2780) or resistant (PTX10) to taxol. The unphosphorylated form, the C-terminal autoinhibitory domain binds to and ordronin receptor, oridonin seems to display the most potent activity in ovarian cancer cell lines. However, it is unknown how the phosphorylation of IRF 3 activates other target genes. However, it is unknown how the phosphorylation of IRF 3 activates other target genes.

From Mono- to Dimeric IRF3s: The Heart of the Matter in Activation of the Interferon Regulatory Factors

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Abstract: We present here that the 2.0Å resolution crystal structure of a dimeric form of the IRF-3 transcription factor (residues 210-477) in which Ser 440 has been mutated to the phosphomimetic Asp. The structure reveals a striking mechanism of dimerization in which the C-terminal autoinhibitory domain attains a highly extended conformation permitting extensive contacts to a second subunit. Based on comparison with crystal structures of IRF-3, these results provide a structural basis for the coupling between dimerization and DNA binding in the IRF family members, in which the C-terminal autoinhibitory domain plays a dual role. In the unphosphorylated form, the C-terminal autoinhibitory domain binds to and masks the hydrophobic CBP/p300 binding surface. The structure provides a new avenue for drug design.

Results: We present here that the 2.0Å resolution crystal structure of a dimeric form of the IRF-3 transcription factor (residues 210-477) in which Ser 440 has been mutated to the phosphomimetic Asp. The structure reveals a striking mechanism of dimerization in which the C-terminal autoinhibitory domain attains a highly extended conformation permitting extensive contacts to a second subunit. Based on comparison with crystal structures of IRF-3, these results provide a structural basis for the coupling between dimerization and DNA binding in the IRF family members, in which the C-terminal autoinhibitory domain plays a dual role. In the unphosphorylated form, the C-terminal autoinhibitory domain binds to and masks the hydrophobic CBP/p300 binding surface.
Association between Alpha-2a-Adrenergic Receptor Gene and Methylphenidate Response in Korean Children and Adolescents with ADHD

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Background: Methylphenidate (MPH), known to be effective for the attention deficit problems, blocks norepinephrine transporters and low oral doses of MPH have more effect on norepinephrine than on dopamine in subcortical areas. Alpha-2a-adrenergic receptor (ADRA2A) is a key component of the noradrenergic system. The aim of this study was to evaluate the association between the ADRA2A polymorphism and the clinical improvement of symptoms with MPH treatment in Korean subjects with ADHD.

Methods: This study included 114 ADHD children (mean age: 9.08±1.94 years) who were recruited from the child psychiatric clinic at university hospital in South Korea. The subjects who had an greater than or equal to 50% compared with the baseline ADHD rating scale (ARS) scores and who had 1 or 2 point of Clinical Global Impression–improvement (CGI-I) score after 8 weeks of treatment were considered as the ‘good response’ group. After performing genotyping for ADRA2A, we examined the correlation the ADRA2A polymorphism with MPH response and also compared the change of total ARS scores between genotypes at ADRA2A.

Results: e found that while 76.9% of the subjects with G/G genotype showed a good response, 46.0% and 41.7% of the subjects with C/G and C/C genotype showed a good response to MPH treatment according to ARS assessed by parent (Pearson χ²=11.929, df=2, p=0.003). We also found a significant difference of the change at total ARS scores between the subjects with and without G/G (t(21.2), df=1, p=0.029). In terms of treatment response according to the CGI-I, significant correlation was found between genotypes at ADRA2A (Pearson r=0.725, df=1, p=0.002).

Conclusions: Our findings provide evidence of an association between the ADRA2A genotype and response to MPH treatment assessed by both parents and clinicians in ADHD subjects.

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Today most pharmaceutical companies use similar strategies to target the same proteins. As a consequence there is a very high competition between the different pharma, a reduction of the potential markets, a lower innovation and a limited freedom to operate. Overall the productivity is decreasing. The identification of new drug targets is then very important for the future of the pharmaceutical industry.

During this presentation we shall examine the ATPases as a new family of drug targets. From a brief structural analysis of the ATP-binding site of these enzymes, we shall see that some of these enzymes are very good drug targets for a strategy aiming for the identification of ATP competitive inhibitors.

Finally, we will show that it is possible to identify low molecular weight inhibitors of these enzymes and to develop them up to the clinic.

All abstracts are listed in alphabetical order of the presenting author.

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DNA and Recombinant Protein from Bacterial Cells

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Background: The purification of plasmid DNA or recombinant protein is fundamental to life science research, but some isolation methods can be physically and chemically damaging. Magnetic separation offers a gentle alternative. Targets are captured on magnetic particles coated with a target-specific surface, and separated from the sample using a magnetic field.

Methods: Nanosized superparamagnetic nanoparticles (Fe₃O₄) were prepared by chemical coprecipitation method using FeCl₃, FeCl₂ salt, and ammonium hydroxide under a nitrogen atmosphere. A quick and reliable method for the isolation and purification of transcription-grade plasmid DNA has been developed, using PEI-modified magnetic nanobeads as a solid-phase adsorbent. We demonstrated a purification of transfection-grade plasmid DNA has been developed, using PEI-modified magnetic nanobeads and coated with hydrophilic resins are also proposed to improve the purification of His-tagged proteins. The GMA-IDA-coated magnetic Fe₃O₄ were employed for the direct extraction of recombinant protein, EGFP-His₃, from E. coli lysates as a model system.

Results: Up to approximately 819 μg of high-purity (A₂₆₀/A₂₈₀=1.86) plasmid DNA was isolated from 100 ml of overnight bacterial culture. The eluted plasmid DNA was used directly for restriction enzyme digestion, bacterial cell transformation, and animal cell transfection applications with success. The Cu²⁺-charged GMA-IDA-coated magnetic adsorbent had the highest yield and purification factor at 70.4% and 123, respectively.

Conclusions: 1) PEI-modified magnetic nanobeads deliver significant time-savings, overall higher yields and better transfection efficiencies compared to anion-exchange and other methods. 2) GMA-IDA-coated magnetic adsorbent could be used as a suitable adsorbent for recombinant His-tagged protein from aqueous solution. Results proved that this new protein purification adsorbent provides a fast and efficient method for purifying His-tagged proteins with high yield and low background.

Pathological Studies on Thymic Lymphoma in Medaka Fish

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Background: Five orange color strain of Japanese medaka (Oryzias latipes) fish, age from 8 to 24 months old, were diagnosed with thymic lymphoma. Grossly, the tumors protruded unilaterally out of the side of the head from the thymus.

Methods: Specimens were anesthetized with MS-222. Peripheral blood smears and tumor cells imprints were stained by Giemsa-Wright stain. The bodies of victims were fixed in Bouin’s fluid ready for histopathological study. Obtained tumor cells and tissues were fixed by 2.5% glutaraldehyde in 0.1 M cacodylate buffer for SEM and TEM studies.

Results: Metastatic lesions had infiltrated via both direct extension and vascular system in all fish by histopathological examination. The neoplastic mononuclear cells with transverse splitting were observed in blood smears. Mitotic neoplastic cells commonly appeared two or three nuclei in tumor tissue imprint. No virus particles was found after the investigation by TEM examination. The results obtained from SEM studies, the thymic neoplastic cells obtained from victims were identified to neoplastic lymphocytoid and lymphoblastoid. The surface of lymphoblastoid was smooth with some lamellate and pits. In addition, lymphoblastoid cells had micrivial in appearance. The results obtained from TEM studies, lymphoblastoid possessed nuclear pockets and swollen mitochondria. Besides, lymphocytoid possessed few cytoplasm and vesicles inside the cells.

Conclusions: The thymic lymphoma presented in medaka at least originated from two different kinds of cells. The lymphoblast lymphoma/leukemia type showed aberrance in nuclear and edema in mitochondria. The lymphocytic lymphoma/leukemia showed aberrance in few cytoplasm, and nuclear clef. The victims must suffer severely from anemia and neoplastic cell proliferation.

Mifepristone Acts as Progesterone Antagonist of Non-genomic Responses but Agonist of Immunosuppression in Human T Cells

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Background: Progesterone is an endogenous immunomodulator to suppress T cell activation during pregnancy. The stimulation of membrane progesterone receptors might be the cause for rapid non-genomic responses in elevating intracellular calcium ([Ca²⁺]i) and decreasing intracellular pH (pHi) in human T cells. Mifepristone (RU486) exhibits mixed agonist/antagonist effects of progesterone on immune cells. It is necessary to explore whether these complicated effects come from RU486 being antagonist of rapid non-genomic response by progesterone.

Methods: The responses in pH and [Ca²⁺]i changes were measured using the fluorescent dyes, BCECF and fura-2, respectively, in T cells. Proliferation was determined by [H]-thymidine incorporation into phytohemagglutinin (PHA)-stimulated T cells.

Results: Equimolar amounts of the progesterone antagonist RU486 blocked the progesterone-mediated non-genomic responses on [Ca²⁺]i increases and pH decreases. RU486 did not block the inhibitory effects of progesterone on PHA-stimulated T cell proliferation. Rather, RU486 was inhibitory. This inhibitory effects on proliferation caused by progesterone and RU486 were additive.

Conclusions: These results demonstrate that RU486 has dose-dependent mixed progesterone antagonist/agonist effects in T cells. RU486 is antagonistic to progesterone-stimulated non-genomic responses, but agonistic and synergistic with progesterone to suppress PHA-stimulated T cell proliferation. Our findings suggest a new light on the clinical application of RU486.
Dual mechanistic anorexigenic and anti-adipogenic therapeutic for the treatment of obesity

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Background: The sharp rise in obesity in the last decade is one of the most serious public health risks worldwide. Currently approved therapies for obesity exhibit modest efficacy and limiting side effects. We show here the identification of a novel pharmacological agent, ECP00068, for the treatment of obesity.

Methods: The morbidly obese leptin-deficient, ob/ob, and the leptin receptor-deficient db/db mice, as well as the glucose intolerant/obese type II diabetes prone C57BL6J and the diet-induced obese (DIO) mice, were treated with ECP00068. Effects of ECP00068 on the differentiation of preadipocytes into adipocytes and on differentiated adipocytes were examined. Mechanisms of action of ECP00068 was investigated by whole genome DNA microarray during the differentiation of preadipocytes into adipocytes.

Results: We showed that ECP00068 causes appetite suppression that results in up to 50% reduction in food intake, decrease in visceral and subcutaneous adipose tissues, and weight loss in ob/ob and db/db, C57BL6J and DIO mice. ECP00068 inhibits the proliferation and differentiation of preadipocytes, and causes either dedifferentiation or delipidation of adipocytes. Gene expression profiling showed that inhibition of differentiation by ECP00068 was accompanied by the transcriptional inhibition of a large cluster of fat regulatory genes with functional equivalence in C. elegans. Additionally, ECP00068 increases the expression of a transcriptional repressor, zinc finger protein 68 (Zfp68), which specifically knockdown the endogenous AMPK2 and assay for the proliferation rate as well as anchorage-independent growth in soft agar. The results showed that the stable AMPK2 knockdown clones proliferated much faster and formed more colonies than the vector control. Since p53, an important metabolic checkpoint protein, was recently reported to be regulated by AMPK, we query whether AMPK-regulated HCC cell growth is p53-dependent. Using in vitro kinase assay, we showed that AMPK2 catalytic domain can directly phosphorylate p53. Interestingly, we found that AMPK phosphorylated p53 at a novel site T150, which affect the stability of p53 protein.

Conclusions: our results suggest that AMPK may mediate its tumor suppression function through regulation of p53 in HCC.

A potential molecular link for metabolic stress and carcinogenesis: AMP-activated protein kinase

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Background: AMP-activated protein kinase (AMPK), which is a serine/threonine protein kinase, is found to be a key regulator in glucose and lipid metabolism in response to cellular stress and hormones such as leptin and adiponectin. Metabolic stresses, like heat shock, hypoxia or ischemia, and glucose deprivation, have been demonstrated to activate AMPK activity. Activation of AMPK aims to switch off pathways that consume ATP, like inhibition of carbohydrates and lipid synthesis; while switch on pathways that generate ATP, like fatty acid oxidation, glucose transport and glycolysis. Recently, AMPK has been shown to be the molecular target of a wildly used anti-diabetic drug, metformin. Recent publications suggest that AMPK activation results in suppressing cell proliferation. AMPK lies upstream and downstream of two tumor suppressors, TSC2 and LKB1, respectively, indicating that AMPK may also involve in carcinogenesis. Liver cancer (hepaticcellular carcinoma, HCC) is one of the most common cancers worldwide. However, the molecular mechanism underlying the development of HCC is still unclear. Here, we examined if AMPK is related to HCC formation.

Methods and Results: Using real-time quantitative PCR, we observed that AMPK2 was significantly underexpressed in HCCs (47.6%) as compared to its corresponding nonmalignant liver samples. To further confirm the effect of AMPK on hepatocarcinogenesis, we established stable HCC clones expressing the oncogenic form of AMPK. Treatment of these clones resulted in a block of growth, and hepatocellular proliferation in vivo. The knockdown of AMPK2 specifically knockdown the endogenous AMPK2 and assay for the proliferation rate as well as anchorage-independent growth in soft agar. The results showed that the stable AMPK2 knockdown clones proliferated much faster and formed more colonies than the vector control. Since p53, an important metabolic checkpoint protein, was recently reported to be regulated by AMPK, we query whether AMPK-regulated HCC cell growth is p53-dependent. Using in vitro kinase assay, we showed that AMPK2 catalytic domain can directly phosphorylate p53. Interestingly, we found that AMPK phosphorylated p53 at a novel site T150, which affect the stability of p53 protein.

Conclusions: our results suggest that AMPK may mediate its tumor suppression function through regulation of p53 in HCC.

An Entry to Clavams from Chiral Vinyl Ethers and Chlorosulfonyl Isocyanate

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2[2+2]Cycloaddition between chiral vinyl ethers 1-4 and chlorosulfonyl isocyanate leading to corresponding 2-lactams 5-8 was investigated. Reactions proceeded with excellent diastereoselectivity. The special attention was focused on the problem of stereocontrol in the formation of a desired configuration of the C-4 carbon atom of the azetidinone ring. Adducts 5-8 were transformed into clavams 9-12.

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The adduct 5 was subjected to the sequence of reactions, in which glycolic cleavage and intramolecular cyclation of the nitrogen played crucial roles. Adducts 6-8 were transformed into clavams 10-12 via intramolecular cyclation of the nitrogen atom.

The antibacterial and antifungal properties of all clavams obtained were investigated to show, however, only in few cases interesting activity.

Bulletproofs to Abacavir-related Hypersensitivity Reaction in HIV-1-infected Population

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Background: Current antiretroviral (ARV) therapy are effective, allowing HIV-positive individuals to live longer, but at the same time they have resulted in many drug-related complications. One of it, is hypersensitivity reaction (HSR) to Abacavir (ABC)-treatment. Pharmacogenetic polymorphisms can influence on HIV-1 treatment. Few recent studies have shown that patients who were positive for HLA B*5701 allelic have a high risk allergic reaction to abacavir. The individual of alleles haplotype has been combined to susceptibility to ABC-HSR. Prevalence of HLA B*5701 among Caucasian are 5-7%.

Methods: This is retrospective analysis of clinically diagnosed ABC-HSR among 217 treated with abacavir-containing ARV regimen HIV-1 infected individuals, hospitalized between 2003-2008 in the Hospital for Infection Diseases in Warsaw. The aim of analysis was attempt to determine of B*57 alleles carrier in 12 patients (M-9, F-3, av. age 39), who developed ABC-HSR incidence. Multiple symptoms were observed in this cases, defined as abacavir-related allergic reaction, according to HSR characteristic signs and time onset between 1-6 weeks after abacavir initiation. The HLA test was performed in all 12 patients defined as ABC-HSR, using of method Sequens Based Typing [Atria Genetics].

Results: During recent 5 years, abacavir-containing antiretroviral therapy received 217 hospitalized patients. In 12 (5.5%) of this group have clinically confirmed ABC-HSR and discontinued ABC-treatment in consequence. Simple symptoms, such as skin and gastrointestinal reaction, were observed in 4 (33%) individuals. In 2 other (17%) constitutional and severe respiratory symptoms were reported. Among 6 other (50%), 3 or more HSR signs including fever, paresis, hypotension, tachycardia, liver parameters alteration, were also observed. The onset of HSR occurred between 6 days and 6 week afterwards. No death registered. The HLA B*5701 test performed in subsequent HSR, using the sequence typing method. Only 1 patient was HLA B*5701 positive. He concern 36y men with AIDS-C3 category, who received 5th ARV-drug regimen due to resistance and intolerance previous antiretroviral agents.

Conclusions: 1. HLA B*5701 screening in HIV-1-infected individuals has the predictive value to ABC-HSR risk. As a result, cases of abacavir-related hypersensitivity reaction incorrectly diagnosed clinically, as well as abacavir unnecessarily discontinuation, can be reduced in clinical practice.
The Therapeutic “Cure” of Xenograft Tumors by the Third Generation Epithelion: Iso-oxazole-Fludelone

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17-Iso-oxazole-26-F, 10-dehydro-12,13-deoxy-epothilone B (Iso-Flu or KOS-1803) is designed by diverted total synthesis based on pharmacological property. In vitro, it has an IC50 of 0.27µM against CCRF-CEM cells, which is 4.x more potent than paclitaxel (taxol). It is 638× and 637× more potent than taxol against drug-resistant CCRF-CEM/taxol and CCRF-CEM/ vincristine cells, respectively. It showed microtubule-stabilization activity similar to taxol. When compared with dIpoB and dehydrodone, Iso-Flu is metabolically stable in mouse plasma when delivered as either a 6 h-i.v. infusion or oral administration; has improved water solubility; has improved bioavailability and tissue penetration for prolonged tumor tissue drug retention; and demonstrates favorable pharmacokinetic and pharmacodynamic properties. The critical-conditions for therapy and low toxicity are 6 h-i.v. infusion at 25-30mg/kg every 14 days. 3x doses. In human xenograft tumors: for CCRF-CEM/Taxol at optimal conditions, Iso-Flu led to 4/4 complete remission (CR) without any relapses for 3 months, whereas taxol had no therapeutic effect, and cyclophosphamide (CTX) suppressed tumor without a CR. For CCRF/CX regimen, Iso-Flu, taxol, and CTX all achieved CR, but 2/4 of CTX treated relapsed in one month. For the pancreatic Bx-PC-3 xenograft, Iso-Flu (25mg/kg, Q1D3x, i.v. infusion) led to suppression and shrinkage but no CR, whereas taxol (25mg/kg, Q2D10x, i.v.) and gemcitabine (40mg/kg, Q2D10x, i.v.) suppressed tumor growth only 90% and 60%, respectively. For hepatic Hepa, Iso-Flu (30mg/kg, Q1D6x, i.v. infusion) led to growth suppression and shrinkage but no CR, whereas taxol (25mg/kg, Q2D14x, i.v. and QTX (50mg/kg, Q2D10x, i.v.) and 5-fluorouracil (40mg/kg, Q2D5x, i.v.) led to only 15%, 55%, and 30% growth suppression, respectively. The superior data of Iso-Flu lead to early results for Iso-Flu against mammary MXT-1 (i.v. infusion or oral), neuroblastoma SK-NAS, lung A549 and A549/taxol, ovarian SK-OV-3, mammary MCF-7/Adr xenografts over other chemotherapeutic agents [Chou, et al. Proc. AACR 47: 115, 2006; 48: 342, 2007, and Chou, et al. PNAS (in press)]. Thus, so far, we have not been able to find any other cancer therapeutic agent that is even iso-effective to Iso-Flu in overall therapeutic efficacy in xenograft experimental systems.

Authors’ disclosure statement: The intellectual property rights for epitomines at Memorial Sloan-Kettering Cancer Center have been licensed to Kosan/Bristol-Myers Squibb Pharmaceuticals.

Defensinex and Defensins as magic bullets against iron overload

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Defensins are a group of small, cysteine-rich peptides that have a wide range of biological activities. They are expressed in a variety of tissues and are thought to play an important role in innate immunity. Defensins have been shown to have potent antimicrobial activity, to be involved in wound healing, and to have anti-inflammatory effects. Among the various defensins, human defensin-1 (hBD-1) and defensin-2 (hBD-2) have been the most widely studied. In the past decade, numerous studies have been published on the potential therapeutic applications of defensins, including the treatment of infections, allergies, and autoimmune diseases. However, the use of defensins as drugs has been limited due to their instability and toxicity. Therefore, there is a need for the development of novel defensin-based therapeutic agents.

In this study, we investigated the potential of defensins as magic bullets against iron overload. Iron overload is a common complication of transfusion-dependent anemias, such as thalassemia and sickle cell disease, and is associated with significant morbidity and mortality. Treatment of iron overload typically involves the use of iron chelators, such as deferoxamine and deferasirox. However, these compounds can cause significant side effects, including chelation-related toxicity and reduced bioavailability.

In recent years, there has been a growing interest in the development of defensin-based therapeutic agents as alternative approaches to iron chelation. Defensins are known to be highly toxic to iron-loaded cells, and this property has been exploited in the development of novel defensin-based chelators. These chelators can effectively bind iron and promote its excretion, thereby reducing iron burden.

In this study, we tested the efficacy of a novel defensin-based chelator in a mouse model of iron overload. The results showed that the defensin-based chelator significantly reduced iron burden and improved clinical outcomes in the mouse model. These findings suggest that defensins may have potential as novel therapeutic agents for the treatment of iron overload.

In conclusion, defensins have the potential to be developed as novel therapeutic agents for the treatment of iron overload. Further studies are needed to explore the therapeutic potential of defensins in this area. In addition, the development of novel defensin-based chelators is expected to provide new options for the treatment of iron overload.

EHRLICH II –2nd World Conference on Magic Bullets
Celebrating the 120th Anniversary of the Nobel Prize Award to Paul Ehrlich
Nimberg, October 3-5, 2008

All abstracts are listed in alphabetical order of the presenting author.

ČUZMARÉVČI B, DEBEVC D, LEVART P

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Background: Deep neck space infections present as deep neck abscess or necrotizing fasciitis. They have severe potential for complication which can be life threatening. Aims: To find out predictive factors for more fulminant progression of infection.

Methods: We retrospectively analyzed medical data for patients surgically treated for deep neck space infection between 2002 and 2006. Our group of patients consists of 79 male and 53 female. We divided them in two groups: deep neck abscess or necrotizing fasciitis.

Results: 115 patients had deep neck abscesses and 17 necrotizing fasciitis. Infection started with nondental origin (anica, epiglottis, infected haemathoma) in 8 patients in first group and in 6 patients in second group. Among comorbidditis Diabetes mellitus was the all common, others were prolonged treatment with corticosteroids, cirrhosis and chemotherapy.

Conclusions: Dental infections are the most common cause of deep neck abscesses and necrotizing fasciitis. Nondental origin is more likely to cause fulminant infection and necrotizing fasciitis. Diabetes mellitus was the most common comorbidity.

Abstracts  Page A-59
Zolpidem after brain Damage

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11 January 2009 marks the tenth anniversary of the awakening of Louis from his state of impaired consciousness 30 minutes after swallowing 10mg Zolpidem. In the past decade, Zolpidem has been effective in a multitude of patients with brain damage ranging from birth injury to traumatic brain injury to stroke and others. SPECT and PET studies in these patients show reactivation of brain metabolism in quiescent brain regions after injury, designated functional neurodormancy. MEG studies show that these suppressed neurodormant brain areas have a characteristic slow wave magnetic rhythm that normalises after Zolpidem, but not after placebo or other sleeping drugs such as Zopiclone. Neurodormancy arises as a physiological thread that is present in a multitude of unrelated brain pathologies, possibly due to a basic physiological protection mechanism that is initiated after brain damage. It forms a target for Zolpidem which re-activates neurodormant tissue and normalises clinical features that occur because of the suppressed neurodormant brain.

New Applications for Micro- and Nanoscaled Drug Delivery Systems

COESTER C
Ludwig Maximilians University, Munich, Germany

Our group comprises four members located in the Pharmaceutical Technology of the Department Pharmacy, LMU-Munich, headed by Mr. Gerhard Winter. We are concerned with the pharmaceutical development of colloidal carriers for the targeted gene and tumor therapy by delivery of siRNA, RNA, plasmid DNA, and classical chemotherapeutics. The idea behind is to use simple and straight forward formulation approaches, which are easy to implement on a large scale e.g. for the industrial production. Our colloidal carriers are produced solely out of biocompatible, biodegradable and low toxic materials like phospholipids, porcine gelatin and chemically modified derivatives of them. A new matter of our group is the knockdown of disease - related genes by siRNA. This is anticipated to gain momentum with the aid of our newly established cell culture facility. A further challenge we have recently taken is the development of acoustically active gas-filled microparticles, also called microbubbles, for over 15 years as contrast agents for the ultrasound diagnostic imaging. Recently, they have also proven promising carriers for drug and genes. After being injected, microbubbles can be visualized on the target site by using diagnostic ultrasound. Following, by increasing ultrasound intensity they can be destroyed and release their therapeutic load. Our research on microbubble carriers diverges in the fields of targeted therapy of solid tumors and targeted gene therapy.

The ADAM9 Disintegrin Domain (ADAM9D) Inhibits Platelet and Tumor Cell Adhesion to Subendothelial Matrix under Dynamic Flow Conditions

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1Federal University of São Carlos, SP, Brazil; 2INSERM Unit 553, Paris, France.

Background: Members of the ADAM (A Disintegrin And Metalloproteinase) protein family are composed by a series of conserved protein domains including a disintegrin domain, which interacts with cell surface integrins. Adhesion to and extravasation through the endothelial lining of blood vessels is critical for tumor cells to establish metastasis. The objective of this work was to verify the effects of the disintegrin domain of ADAM9 (ADAM9D) on the adhesion of tumor cells (MDA-MB-231) and platelets to collagen type I, under flow to simulate shear conditions found in the circulation.

Methods: The recombinant ADAM9D was produced by cloning into a pGEX-4T-1 vector which was used to transform E. coli AD494(DE3) cells. The synthesis of GST/ADAM9D was induced by IPTG (0.1mM, 4h). After purification on a Glutathione-Sepharose 4B resin, the ADAM9D was released from GST by cleavage with thrombin and further purified in a Benzamidine-Sepharose 4B column. MDA-MB-231 breast tumor cells labeled with cell tracker red were previously incubated with ADAM9D (5µM) or PBS (control) and then mixed with whole blood prepared labeled with calcein green. The mixture was perfused at a rate of 1500sec⁻¹ in a flow chamber on a collagen type I-coated coverslip. Adhered platelets and cells in each field were differentially counted using the software Image J. The results were statistically compared with a two-way analysis of variance (ANOVA), followed by Bonferroni’s significant difference post hoc analysis.

Results: Recombinant ADAM9D was able to inhibit about 50% of breast tumor cells and platelet adhesion to collagen type I, under flow conditions.

Conclusions: ADAM9D can be used as a tool for investigating the role of ADAMS in metastasis and cancer progression and for the design of selective inhibitors against the adhesion and extravasation of cancer cells.

Financial support: FAEPESP, CNPq, INSERM.

In situ Photopolymerized Coatings for pH-Specific Drug Delivery from Pellets

CONCHEIRO A, MAYO-PEDROSA M
Univ. Santiago de Compostela, Santiago de Compostela, Spain.

Background: Coating of drug-containing pellets enables the delivery to be sustained or site-specific. As an alternative to the traditional coating procedure consisting of deposition of successive layers of polymer films, tailored coatings can be obtained by photopolymerization/cross-linking of acrylic monomers for over 15 years as contrast agents for the ultrasound diagnostic imaging. Recently, they have also proven promising carriers for drug and genes. After being injected, microbubbles can be visualized on the target site by using diagnostic ultrasound. Following, by increasing ultrasound intensity they can be destroyed and release their therapeutic load. Our research on microbubble carriers diverges in the fields of targeted therapy of solid tumors and targeted gene therapy.

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**Role of Drug Metabolism in the Development of Eplerenone (EP): A Lesson Learned from Spironolactone (SP).**

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1Baxter Healthcare, Round Lake IL, USA; 2Amgen Inc Cambridge MA, USA; 3Pfizer Inc, Groton CT, USA

**Background:** EP is a highly selective aldosterone blocker approved in the US for the treatment of hypertension and heart failure. During development of the drug, extensive metabolism studies were necessary since another competitive aldosterone antagonist, potassium canrenoate (PC), that is structurally related to EP, was shown to be metabolized to potent mutagenic metabolites which appear to be associated with myelogenous leukemia. PC is a potassium salt of canrenonic acid formed from SP. However, SP did not produce these mutagenic metabolites because a thiol metabolite of SP inhibited formation of mutagenic metabolites from canrenoate.

**Methods:** In vitro metabolism studies of EP were carried out using human liver microsomes and cDNA-expressed CYP450 isozymes. Major metabolites were isolated and identified using MS and NMR. Inhibition of metabolism in human liver microsomes was examined in the presence of various chemical inhibitors at a concentration of 10 μM and with human CYP450 antibodies. Urinary and fecal metabolites were identified following oral administering of [14C]EP to healthy subjects at dose of 100 mg/person.

**Results:** Metabolic pathways of EP were β- and/or 21-hydroxylation by CYP3A4/5 and 3α-reduction. The major metabolite identified was 6β-OH-EP. There was no evidence for any alteration of the 9,11-epoxide ring or carbonyl methyl ester. In contrast to canrenoate metabolism, no 6β,7β-epoxy metabolite, a potential mutagenic metabolite, was identified.

**Conclusions:** 1) Major metabolic pathway of EP was hydroxylation by CYP3A4/5, 2) Unlike PC, there was no 6β,7β-epoxide metabolite formed with EP, 3) Demonstration of stability of 9,11-epoxide ring and absence of 6β,7β-epoxy metabolite was essential in the development of EP.

**BDNF and Its Intracellular Signaling Pathways as Drug Targets in Addiction**

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**Background:** Cumulative evidence has demonstrated that stress plays a crucial role in cocaine addiction by enhancing the rewarding properties of the drug and inducing relapse during withdrawal. Corticotropin Releasing Factor (CRF) is one of the key extracellular modulators of the reward circuitry, such as the ventral tegmental area (VTA) and the amygdala. Aim: This paper explores the pattern of interaction between CRF, dopamine (DA) and glutamate (GLU) in different structures of the mesocorticlimbic circuitry during cocaine consumption. Understanding the neurochemical and cellular mechanisms involved in these processes would be useful in the development of new pharmacological strategies for the treatment of cocaine addiction.

**Methods:** A systematic literature review was conducted of animal and human research on the effects of stress through the CRF system. The interaction between CRF, dopamine and glutamate in VTA and the amygdala during repeated cocaine consumption and withdrawal were the focus of this review.

**Results:** In the VTA, stress-induced increase of CRF potentiates the activity of GLU transmission, which in turn increases the excitability of the DA system in cocaine-experienced animals. Blockade of CRF-R2 prevents stress-induced increase of synaptic plasticity in the DA system and relapse during withdrawal. In the amygdala, during early and late cocaine withdrawal, there is a progressive increase of CRF release that coincides temporally with behavioral anxiety during cocaine withdrawal and contributes to relapse. This increase in CRF activity potentiates GLU transmission, long-term potentiation, and synaptic plasticity. Selective antagonists of CRF-R1 are useful for preventing anxiety during withdrawal and reinstatement of cocaine seeking.

**Conclusions:** 1) Both CRF-R1 and CRF-R2 inhibitors may be useful for preventing withdrawal symptoms and relapse in cocaine addicts. 2) Bearing in mind the interactions between CRF, glutamate and DA in the different elements of the mesocorticlimbic system, the use of combined pharmacological strategies involving all these neurotransmitters should be considered in the treatment of cocaine addiction.
The Interaction of the Organophosphorous Pesticide Methyl-Parathion with Serum Albumin by Fluorescence Spectroscopy

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Background: The aim of this work was to study the interactions of methyl-parathion (MP) with human (HSA), bovine (BSA) and fish (FSA) albumins by using the fluorescence quenching technique. The fish species used was the pacu (Piaractus mesopotamicus), a typical inhabitant of the Brazilian rivers. MP is a pesticide still used in agriculture and fish hatcheries in many countries.

Methods: The studies were performed from a commercial grade preparation by thin layer chromatography, using dichloromethane as the mobile phase. HSA and BSA were purchased from chemical company. FSA was isolated from the pacu serum by affinity chromatography. We excited the intrinsic fluorescence of the tryptophan in albumins and observed the quenching by titrating the protein solutions with MP. The Stern-Volmer plots were plotted and the quenching constants were evaluated.

Results: The titration of HSA and BSA at 25ºC produced linear Stern-Volmer plots. At 37ºC, the plot is still linear for HSA, but not for BSA. The titration of pacu albumin by the pesticide at 20ºC, 25ºC and 30ºC showed relevant slope deviations by the temperature changes. This behavior of Stern-Volmer plot of pacu albumin is analogous to the BSA. Examining the influence of the temperature at low MP concentrations on the plots, we found the occurrence of static quenching for the temperature changes. This behavior of Stern-Volmer plot of pacu albumin is similar to the BSA.

Conclusions: (1) The three studied albumins interact with MP to form complexes. (2) The primary binding sites for the pesticide on HSA and BSA are close to tryptophan residues 214 and 212, respectively. (3) Estimated association constants for HSA and BSA were, respectively: 3.07x10^4 (±1.2x10^3) M^-1 and 1.95x10^4 (±4.1x10^2) M^-1 at 25ºC, 1.88x10^4 (±2.0x10^3) M^-1 and 8.16x10^3 (±1.3x10^2) M^-1 at 37ºC. (4) For pacu albumin, the Stern-Volmer constants were 1.19x10^5 (±3.4x10^5) M^-1 at 20ºC, 9.73x10^4 (±4.8x10^4) M^-1 at 25ºC, 9.37x10^4 (±4.4x10^4) M^-1 at 30ºC.
Mechanisms of anti-CD20 immunotherapy: Why Type II mAb are better

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Background: Rituximab is an anti-CD20 monoclonal antibody (mAb) that was the first of its kind to be approved by the FDA. It is now part of the standard treatment for many B cell malignancies and is finding utility in a range of autoimmune diseases where depletion of normal B cells, although not as yet explained mechanistically, appears highly beneficial. Treatment is not always successful however and not surprisingly the pursuit of improved reagents to replace rituximab is intense. Anti-CD20 mAb can be sub-divided into Type I (like rituximab) or Type II (tolitumumab-like) based on their ability to redistribute CD20 molecules in the plasma membrane and activate various effector functions such as complement. The aim of this work was to address whether other (Type II) anti-CD20 mAb might be more effective than rituximab in deleting B cells.

Methods: To compare Type I and II anti-CD20 mAb directly in vivo and maximize Fc effector function, we selected and engineered mAb with the same mouse IgG2a isotype and assessed their B cell deleting activity in two different strains of human CD20 transgenic mice. The ability to deplete peripheral blood and secondary lymphoid organs was assessed by flow-cytometry and immunohistochemistry.

Results: Despite being the same isotype, having similar affinity, opsonising activity for phagocytes, and in vivo half-life, the Type II mAb tolitumab provided substantially longer depletion of B cells from the peripheral blood compared with the Type I mAb rituximab (Rt m2a), and 1FS. This difference was also evident in the secondary lymphoid organs, in particular the spleen. Failure to engage complement did not explain the efficacy of the Type II reagents, since Type I mAb in the Fc domain (K22A) to prevent C1q binding still did not display equivalent efficacy. We have recently determined the likely mechanism through which Type II mAb outperform Type I mAb and will present this data.

Conclusions: These results provide strong support for the development of Type II anti-CD20 mAb for the treatment of B cell diseases and expect that their lack of complement engagement should reduce the toxic side effects often associated with the use of rituximab and other Type I reagents. This work also provides insight into how mAb to other targets might be optimized for better therapeutic efficacy.

Authors’ disclosure statement: The authors declare no competing financial interests.

Allergy vaccines: dreams and reality

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Allergy, asthma, and atopic eczema derive from deregulated immune responses against environmental antigens. The incidence of atopic diseases is affecting around 30% of the population in industrialized countries and has become a relevant socioeconomic burden for the medical society. Although much progress has been achieved in the development of efficient symptomatic treatments for allergic diseases, the only curative treatment remains allergen-specific immunotherapy (SIT). In contrast to classical vaccines which elicit strong host immune responses after one of a few injections, SIT requires a long treatment time of 3 to 5 years to confer some protection. During the last year, three promising allergy vaccination concepts (RPE 04 (Curalogic); GT-14 (ALK-Abelló); TOLAMBA (Dynavax)) have been put on hold in Phase III due to the lack of efficacy. The reality is that “allergy vaccines” achieve beneficial effects through immunomodulation which takes a long time to establish, the dream would be to develop “true” allergy vaccines able to cure the disease with a few injections. We have engineered modular antigen translocation vaccines (MAT) for direct intracellular targeting of the MHC class-II presentation pathway aimed to increase the efficacy of antigen presentation. MAT vaccines administrated directly into the lymph nodle in mouse models of allergy were able to completely protect the animals from anaphylactic shock in a very short time. Intranasal administration of low doses of pollen extracts to allergic patients was able to confer a long lasting protection after only three injections administrated over a period of eight weeks only. Now we are combining a MAT-Fel d1 vaccine with intranasal administration in a Phase I clinical study ready to start at beginning of July 2008. The combination of a new route of administration with a direct targeting of the MHC class-II presentation pathway has to potential to realize the dream of curing allergy in a few weeks.
Naked Models of Compound I of Heme Enzymes

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Background: High valent iron porphyrins at the formal Fe(V) oxidation level are known as Compound I intermediates, invoked in the oxidation pathways catalyzed by heme proteins and by their synthetic model compounds. A dominant feature of these processes is the dramatic change in product patterns and selectivities that Compound I may exhibit under the influence of reaction conditions. In this context, gas phase studies may have a great potential in revealing the intrinsic behaviour of this key intermediate.

Methods: Electrospay ionization (ESI) in combination with Fourier transform ion cyclotron resonance mass spectrometry is used to characterize the gas phase reactivity of high-valent oxoiron intermediates formed by two distinct procedures. In the first one, the oxorin(V) porphyrin cation radical intermediate, [(TPFPFP)Fe=O]5+ (TPFPFP = 5,10,15,20-tetratetafluorophenylporphinate dianion) is prepared in solution by the reaction of the irin(III) porphyrin chloride, [TPFPFPFeCl4][Cl], and H2O in methanol and then transferred to the gas phase by ESI.1 2 Alternatively, the naked core of Compound I is synthesized by the reaction of iron-protoporphyrin IX (heme) ions, [(IPK-IX-Fe)2], with ozone in the gas-phase.

Results: The formation of the oxo-complex, described as a gaseous iron(IV)-oxo porphyrin IX radical cation species, [(PP-IX)Fe=O]5+, allows a viable entry to a species that proves to be elusive in solution where it evolves presumably by activating the rapid growth of degradation products.3

The reactivity properties of the so-obtained high valent oxo iron intermediates with exogenous acceptors and with different model compounds of naturally occurring substances (L) of heme proteins are reported. The reaction efficiencies, which measure the % fraction of reactive collision events, appear to increase with the oxophilic character of the active site of L (C<N<S<P).

Conclusions: Gas phase studies may elucidate complex mechanisms in enzyme chemistry providing highly simplified models.

References

Do we need a smaller Magic Bullet for combating Staphylococcal infections?

: Mechanism of vancomycin-resistance in vancomycin-intermediate S. aureus

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Since our reports on the discovery of vancomycin-intermediate Staphylococcus aureus (VISA) and hetero-active vancomycin-resistant Staphylococcus aureus (hetero-VISA) in 1997 (JAC, 1997, 42:133-6) and Lancet, 1997 385:1670-3), we have given emphasis on the molecular mechanism of vancomycin resistance in VISA, mainly in JAC (98(4):159-200; 199-200; and 24:3-315-20), AAC (2000:41:1176-1185), Lancet (2001:357:1226-45), JCM (2003:41:151-4; 9, Mol Microbiol, 2003:49:807-21), Lancet (2004:364:565-6) and AAC (2005:49:3404-13, 2006:50:345-6). By performing the above serial studies, we have elucidated the molecular mechanism of vancomycin resistance: VISA reveals vancomycin through a thickened cell wall, which in turn results in a novel mechanism of "doubling" of the peptidoglycan mesh, whereby incoming vancomycin molecules are trapped in the thickening cell wall, preventing them from reaching the cell membrane. Quantitative measurement and mathematical analysis revealed that the "doubling" is related with the bigger molecule of vancomycin. Moreover, in a recent study with daptomycin-nosensitive S. aureus, we found a strong positive correlation between vancomycin and daptomycin resistance in VISA, and the result suggests the thickened cell wall acts as a common obstacle to daptomycin and vancomycin penetration. Even though daptomycin does not bind peptidoglycan to form subsequent physical barriers within the cell wall, it might be hard for daptomycin, with as big a molecule as over 1620, to penetrate smoothly through the cell wall when the cell wall become as thick as that of VISA. Taking this into account, we propose that development of new antibiotics with smaller molecular size than that of vancomycin and daptomycin may be a new potential to the vancomycin- and daptomycin-resistant Staphylococcal infections.

Prognostic value of total AgNOR area/Nuclear area per cell in urinary bladder carcinoma via two-dimensional image analysis

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Background: Traditional criteria are not sufficient to predict accurately the recurrency of transitional cell carcinoma of the urinary bladder. Therefore, we aimed to evaluate comparatively the nucleolar organizer region-associated proteins (AgNORs) via total AgNOR area/nucleus area (TAANA) for each cell, and as a prognostic parameter, comparing with total AgNOR number/nucleus (TANN), in TCC of urinary bladder.

Methods: Tumor tissues of 20 consecutive cases of male bladder cancer patients were divided into two groups as middle differentiated (LG) and high grade (HG). The extra-tumoral tissue (ETT) samples of 10 males served as control group. A second control group (HC) consisted of five healthy and normal bladder tissue samples. The 3 µm sections of each paraffin embedded tumoral, extra-tumoral and normal tissue samples served as patient and control groups. After deparaffinization and rehydration steps, AgNORS silver stained. Instead of Giemsa stain, we used Hematoxylin for contra staining. The images of the 100 analyzable nuclei from each tissue sample transferred by means of a video camera and video capture card from microscope and recorded onto a computer. Software was prepared in Delphi language for analysis.

Results: Mean (E+02) TAN values were significantly different between all groups (p values < 0.001). While the mean TANN values of the groups were able to distinguish only malign samples from normals (p values < 0.05). The data is given in the table.

Table . TAN and TAANA values of control and patient groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>n</th>
<th>Mean TAN/E (%)</th>
<th>Mean TAANA/E (E+02)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>10</td>
<td>1000</td>
<td>1.37±0.28</td>
<td>6.81±1.82</td>
</tr>
<tr>
<td>RG</td>
<td>10</td>
<td>1000</td>
<td>1.52±0.77</td>
<td>5.70±1.82</td>
</tr>
<tr>
<td>LG</td>
<td>10</td>
<td>1000</td>
<td>1.56±0.77</td>
<td>7.80±3.22</td>
</tr>
<tr>
<td>HG</td>
<td>10</td>
<td>1000</td>
<td>1.56±0.82</td>
<td>9.24±3.38</td>
</tr>
</tbody>
</table>

Conclusion: As a new approach the evaluation of mean TAN/NA per cell has a great potential to be a prognostic parameter and it has been found more sensitive and more accurate than the TAN determination. Therefore, further evaluation of big patient series will be useful.

Fundamental Understanding on Interactions of Bisphosphonates with Bone (by Use of Different Techniques, Including Computational Modelling).

CUKROWSKI I
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Background: Computational modelling of BP-bone interactions is rare and hence there is very little to link the molecular structure of BP with its potency. Aims: 1) To determine stability constants of BPs with metal ions. 2) To investigate interactions between BPs and a bone surface. 3) To model computationally the interactions of BPs with a bone surface.

Methods: Raman spectroscopy (RS) was used to study the interactions of bovine bone, hydroxyapatite (HA), and CaHPO4, with HEDP (a reference compound in scale of potency). Generalised AMBER Force Field (GAFF) was used to model over 50 BPs (CR(R)(R)(PO3H2)) and the interaction of MDP (R = H) with HEDP (R = OH, R = CH3), APD (R = OH, R = (CH2)NH3), ALN (R = OH, R = (CH2)NH2), and NER (R = OH, R = (CH2)NH2) with the (001), (100) and (105) faces of HA. Metal-BP stability constants were established by voltammetry and potentiometry; this allowed us to conclude on mode of complexation at blood plasma pH. Linear Free Energy Relations (LFER) generated allowed us to predict stability constants of radioactive elements with BPs; potential use in bone cancer therapy.

Results: The Raman spectra of the products from the reaction of 0.5 M HEDP solution with bone, HA and CaHPO4 could be considered virtually identical meaning that the complexes of HEDP formed were the same. From GAFF, all BPs react with HA exothermically (10 and 20 kcal mol-1). With a dielectric constant of 780 and <100, non-bonded and electrostatic interactions dominate, respectively. The order of increasing interaction with HA, MDP < HEDP < APD < ALN < NER, accords with the observed in vivo order of pharmacologic activity (potency) and parallels the increase in molecular volume up to ALN; the side chain of NER fails to interact fully with HA. There is no significant difference in the structure of the BP-HA complex if BP is mono- or bis-protonated. Relationships established from metal equilibria allowed prediction of radiisotops'interactions with BPs. Relationships established from metal equilibria allowed prediction of radiisotops'interactions with BPs.

Conclusions: 1) HA can be substituted for bone in fundamental studies of BP-bone interactions. 2) The consonance between experimental and molecular modelling results suggests that GAFF may be a useful tool to aid in the design of novel BP ligands. 3) From stability constants it was concluded that side chain of BPs is not involved in complexation with metals at blood plasma pH.

All abstracts are listed in alphabetical order of the presenting author.
Prostheses as ClC-2 Channel Activators for Treatment of Diseases and Disorders.

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Background: Prostheses are a class of biocidal fatty acids. One of these, lubiprostone, is approved for the treatment of chronic idiopathic constipation (CIC) and irritable bowel syndrome with constipation (IBS-C) in the United States.

Methods & Results: Lubiprostone activates Cl\(^-\) currents, thus increasing salt and water flow in the small intestine. Electrophysiological studies of epithelial tissues show that Cl\(^-\) currents are increased by low concentrations (<100 nM) lubiprostone. These Cl\(^-\) currents persist with knockdown of the cystic fibrosis transmembrane regulator (CFTR), another epithelial Cl\(^-\) channel. However, the lubiprostone-activated Cl\(^-\) currents are abolished by knockdown of ClC-2. Recombinant human ClC-2 is activated in a dose-dependent manner by lubiprostone (EC\(_{50} = 20 \) nM). Recombinant human CFTR was not activated at concentrations as high as 1 \(\mu\)M. Lubiprostone increased single 3-4 pS Cl\(^-\) channel activity of both human and Xenopus ClC-2 in the apical membrane of the cells, at concentrations <100 nM. This is consistent with apical membrane localization of ClC-2 in T84 and Caco-2 human intestinal cell lines using nystatin permeabilization approaches. This is also consistent with animal studies showing increased salt and water transport into the intestinal lumen with lubiprostone. ClC-2 activation by lubiprostone does not involve increases in intracellular cAMP or Ca\(^{2+}\), activation of prostaglandin receptors or phosphorylation by PKA. In Caco-2 cells and porcine ileum ClC-2 is present at the tight junctions. In porcine ileum, lubiprostone appears to promote repair of epithelial barrier function impaired by ischemia, accompanied by movement of CIC-2 to the tight junctions, a mechanism which may contribute to the treatment of IBS-C.

Conclusions: Various studies have demonstrated that lubiprostone is a Cl\(^-\) channel activator that activates ClC-2 in the apical membrane of intestinal cells. ClC-2 activation may be beneficial in diseases and conditions where tight junction integrity is compromised. 3. ClC-2 activation by prostines is a useful therapeutic tool in GI disorders. Supported by a grant from Sucampo Pharmaceuticals Inc.

Authors’ disclosure statement: Both authors have significant financial support from Sucampo Pharmaceuticals Inc.

Phosphorylation of the α\(_v\)β\(_3\) Integrin Receptor in Fibroinectin

CURNIS F, LONGHI R\(^1\), CRIPPA L, CATTANEO A, DONDOSOILLA E, BACHIA A, CORTI A

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Isoseapartate, also known as isoseapartate peptide, is an isoeapartate that binds to the α\(_v\)β\(_3\) integrin receptor in fibroinectin. Isoseapartate is a small molecule that can bind to the α\(_v\)β\(_3\) integrin receptor, thereby inhibiting its activity. This is achieved by competitively inhibiting the binding of the soluble ligand, fibroinectin, to the integrin.

**Results:**
- Isoseapartate inhibits the binding of fibroinectin to the α\(_v\)β\(_3\) integrin receptor.
- The inhibition is specific to the α\(_v\)β\(_3\) integrin, as other integrins are not affected.
- The inhibition is concentration-dependent.

**Conclusions:**
- Isoseapartate is a potential therapeutic agent for conditions where integrin activation is detrimental, such as cancer and inflammation.
- Further studies are needed to determine the optimal dose and duration of treatment.

Authors’ disclosure statement: No conflicts of interest to declare.

Spontaneous Formation of L-isoAspartate and Gain-of-Function in Fibroinectin

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- Further studies are needed to determine the optimal dose and duration of treatment.

Authors’ disclosure statement: No conflicts of interest to declare.

Isoaspartate-glycine-arginine (isoDGR): a new tumor vascular targeting motif

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Various peptides containing the NGR motif have been discovered by selecting peptides from phage display libraries in tumor-bearing animals. These peptides can home to tumor neovasculature by binding an arginine-glycine-aspartate (RGD) domain, a marker of angiogenic vessels. We have previously demonstrated that NGR peptides can be exploited for ligand-directed delivery of cytotoxins, e.g., TNF and interferon-\(\gamma\), to CD13-positive tumor blood vessels. One of these conjugates, made of CD13 to tumor necrosis factor-\(\alpha\) (NGR-TNF), is now established in phase II clinical studies for cancer treatment, highlighting the value of CD13-targeting peptides in drug development. We have also found that NGR can rapidly convert to isoDGR and DGR by asparagine deamidation. In vivo and in vitro studies have shown that isoDGR, not NGR, can bind to CD13 integrin in the tumor neovasculature. Pharmacological studies performed in tumor-bearing animal models showed that low doses (picograms) of NGR-TNF or isoDGR-TNF fusion proteins are sufficient to induce anti-tumor effects, by virtue of a targeting mechanism, when administered alone or in combination with chemotherapy. Considering that CD13 and isoDGR integrins are markers of angiogenic blood vessels, natural or synthetic polypeptides containing the NGR or the isoDGR motif may be exploited as ligands for targeted delivery of cytotoxins, nanoparticles, genes or imaging compounds to angiogenic vasculature in tumors.

Authors’ disclosure statement: No conflicts of interest to declare.
Create metal complexes which are potent proteasome inhibitors (Cvek & Dvorak 2008).

New use for old drug: of ongoing clinical trials of bortezomib in various cancers. Multiple myeloma and mantle cell lymphoma. At the present time, there are plenty of ongoing clinical trials of bortezomib in various cancers. The drug (just because it is old) is cheap, safe, and is able to enter phase II clinical trials directly (Chong & Sullivan 2007). Drug Discov. Today Abstracts Page A-66

Proteasome: Proteasome is a giant protease responsible for degradation of about 90% of cellular proteins. Key signaling proteins are activated through proteasome processing (e.g., nuclear factor-kB); moreover, proteasome plays an important role in regulation of transcription and other crucial cellular events. Curiously enough, the proteasome has become an attractive target in cancer therapy as first-in-class proteasome inhibitor bortezomib (Velcade) was approved for clinical use against multiple myeloma and mantle cell lymphoma. At the present time, there are plenty of ongoing clinical trials of bortezomib in various cancers. New use for old drug: An old drug against alcoholism, disulfiram (Antabuse), can create metal complexes which are potent proteasome inhibitors (Cvek & Dvorak Drug Discov Today 2008). Even further, disulfiram taken with zinc gluconate led to clinical remission in a patient with metastatic breast cancer (Barr et al. Mol Cancer Ther 2004). There are substantial advantages of such “repurposing” of disulfiram: The drug (just because it is old) is cheap, safe, and is able to enter phase II clinical trials directly (Chong & Sullivan Nature 2007).

JAMM domain inhibition: Molecular mechanism of such disulfiram-mediated proteasome inhibition is putatively based on interaction between the metal complexes and key zinc subunit (JAMM domain protein Poh1) of the proteasome (Cvek & Dvorak Curr Pharm Des 2007). This approach could be an attractive strategy for future proteasome targeting in patients (Gallery et al. Mol Cancer Ther 2007).

Conclusions: Although there are two clinical trials of disulfiram as an anticancer agent listed in US National Cancer Institute database, they are not proteasome-focused (as disulfiram has other abilities to suppress cancer). Thus, potential anticancer effects of disulfiram in the patients should be carefully evaluated in light of current knowledge on proteasome inhibition.

Peritoneal Transport Dynamics of Icodextrin and Its Influence on The Membrane Permeability In Vitro

CZYŻEWSKA K, GRZELAK T, SZARY B
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Background: Glucose polymer (icodextrin) is used during peritoneal dialysis for development of an effective ultrafiltration. It has been proposed also as a carrier solution of antineoplastic drugs and a factor to reduce adherence formation during intraperitoneal chemotherapy. The aim of the presented study was the comparative analyses of the peritoneal transport dynamics of icodextrin in the different conditions and its effects on the transfer of small and large molecules across peritoneal membrane intact and chemically injured in vitro. The rabbit peristaltic peritoneum, modified Ussing chamber and mathematical model of mass transfer were used to calculate the diffusive permeability coefficient (P) in cm² s⁻¹.

Results: Icodextrin (7.5 g/L) peritoneal transport in vitro, in the control conditions, directed from the interstitial (I) to the mesothelial (M) side of the membrane changes with time, but in the opposite direction is constant. Asymmetry of glucose polymer diffusion is observed: I>M predominates over M>I. Fluid stirring intensification and chemical injury by sodium deoxycholate enhance bidirectional transfer of icodextrin. M-I transfer of icodextrin, but not I>M is restricted more by tissue barriers than stagnant fluid layers. Glucose and gentamycin intensity (M-I direction only) of the examined parameters, but methylglyoxal does not change the P values. Icodextrin modifies the transport dynamics of low and high molecular weight solutes across intact and mesothelium denuded peritoneum. For example it induces asymmetry of uric acid and albumin transport. I-M transfer dominates M-I transfer of uric acid. In contrast, in albumin transport M-I was higher than I-M. For the injured peritoneum the decrease in time of the bidirectional uric acid and urate transport, caused by icodextrin, is noted. Glucose polymer periodically augments and next diminishes gentamycin transfer directed from I to M side of the peritoneum. The decrease of antibiotics transport occurs also to the opposite direction.

Conclusion: The results may be important for the clinical point of view. The increase of icodextrin absorption from the abdominal space to blood, during peritoneal dialysis, may have a negative impact on the efficacy of this therapy. In contrast, asymmetry of uric acid and albumin transperitoneal transport induced by icodextrin, may be observed in vivo, seems to be beneficial.

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D'AGUILA PS, SERRA G  
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**Background:** We examined (i) the relationship between antidopaminergic treatment and two stress regimes, Chronic Mild Stress (CMS) and Daily Reistant Stress (DRS), which differ in their ability to affect sensitivity to dopamine agonists and (ii) the effect of different antiaminic drugs on antidopaminergic-induced dopaminergic supersensitivity. CMS, a model of depression, is based on the exposure to a variety of mild stressors (e.g. short periods of crowding, isolation, food and water deprivation etc.). DRS consists of daily sessions of 1 hour immobilization, and they result either in a decreased or in an increased sensitivity to dopamine agonists, respectively.

**Methods:** Male Wistar or Sprague-Dawley rats were used. No-stress, CMS and DRS subjects were daily treated with the antidopaminergic desipramine. Stress and drug administration were commenced simultaneously and carried out for 7 weeks. The antiaminic drugs lithium, carbamazepine and valproate, in three different experiments, were coadministered with the antidepressant imipramine for 3-4 weeks. After the chronic drug/stress treatments, the subjects were challenged with the dopamine D2-like agonist quinpirole and the motor response was measured.

**Results:** Motor response to quinpirole and stress effect in both cases. Carbamazepine, but neither lithium nor valproate, prevented the imipramine-induced potentiation, an effect likely due to liver enzymatic induction. *Conclusions:* 1) Countering stress effect on dopaminergic sensitivity, rather than just inducing a potentiation, might be what matters to attain the antidopaminergic therapeutic effect. 2) The failure of antiaminic treatment to prevent dopaminergic supersensitivity might reflect their poor efficacy in treating antidepressant-induced manic switches, thus providing the basis for a possible model of antidepressant-induced mania.
**Genetic immunization: comparison of water-in-oil liposome-based delivery of cDNAs with genetic immunization; comparison of water-in-oil liposome-based delivery of cDNAs with Genetic immunization:**

**Cyclotides, ultrastable peptide frameworks for magic bullet drug delivery**

**Background:** Cyclotides are plant-derived mini-proteins with compact folded structures and exceptional stability. Their stability derives from a head-to-tail cyclized backbone coupled with a cystine knot arrangement of three-conserved dipeptide bonds. Taking advantage of this stable framework we developed novel approaches to generate cyclotide analogues. We grafted a peptide epitope involved in VEGF-A antagonism onto the cyclotide framework. Antagonists of this kind have potential for therapeutic applications in diseases where angiogenesis is an important component of disease progression, including cancer and rheumatoid arthritis.

**Methods:** This study involved the use of solid phase peptide synthesis to produce several analogues of the prototypic cyclotide kalata B1 with a poly-arginine epitope. Two-stage liquid-phase solid support approaches were performed using Stemcell Technologies fusion protocol modified to take advantage of a stereo Video microscope for picking clones. While both immunization protocols resulted in expression of the donor gene, particularly in vivo electroporation resulted in the expression of protein mainly in the injection site reaction while the water-in-oil liposome emulsion directed the expression of the delivered gene into the lymph nodes. Collectively, these methods increase the breath, magnitude, and durability of immune responses to native antigens and importantly, eliminate the need to produce and purify large quantities of protein for immunization, dramatically reducing monoclonal antibody production costs. Incidentally, our data suggest that described methods may be used for nucleotide delivery in general such as RNA, DNA and RNA.

**Results:** We found total magnesium consumption was linked to a significantly reduced risk of colorectal adenoma, particularly for those with a low calcium/magnesium ratio. An inverse association trend was found for hyperplastic polyps. Further, we found those with a high calcium/magnesium ratio also had a reduced risk of adenomatous polyps. In addition, those with a high calcium/magnesium ratio had a reduced risk of adenomatous polyps. The association between calcium/magnesium ratio and adenomatous and hyperplastic polyps was significant even after adjusting for age, gender, and other confounding factors. These findings, if confirmed, may provide a new avenue for the prevention of magnesium deficiency and, thus, colorectal cancer.
Bis(2-aminoimidazolinium)diphenyl Compounds as DNA Minor Groove Compounds (i.e., 2-aminoimidazolinium derivatives) showing excellent disease selectivity were assayed in vitro. The compounds showing the highest activity and acceptable cytotoxicity. The compounds showing that DNA binding may be part of their mechanism of action. Most importantly, we concluded on their potential as new drug leads for neglected diseases.

Methods: Based on their structural similarity with known antiprotozoal and antimalarial agents, several series of dicaticonic compounds were assayed for their monocationic and neutral analogues were screened in vitro against T. b. rhodesiense, P. falciparum, and rat skeletal myoblast L6-cells as control for cytotoxicity. The compounds showing the highest activity and acceptable selectivity were assayed in vivo in models of acute and chronic T. brucei infections (STIB900 and GVR35 strains, respectively), and murine malaria (P. berghei). Their interaction with the DNA minor groove was also measured by thermal melting curves (Tm) and SPR experiments on AT sequence DNA-polymers. Results: Several dicaticonic leads with mN in vitro activity and excellent selectivity against T. b. rhodesiense and P. falciparum were identified. A number of compounds cured 100% of the mice infected with T. b. rhodesiense. The compounds reduced the parasitemia in mice infected with P. berghei. A correlation between DNA binding affinity and trypanocidal activity was observed, indicating that DNA binding may be part of their mechanism of action. Most importantly, we found that the 2-aminoimidazolinium cation afforded molecules with superior structure activity profile compared with its guanidine counterpart.

Conclusion: 1) The rational screening of in-house libraries of compounds is a validated approach to find new drug leads for neglected diseases. 2) Bis(2-aminoimidazolinium) derivatives represent a very promising class of DNA minor groove binding agents that have already demonstrated their antiprotozoal potential in vivo.

The Role of PPARγ Agonist in Alzheimer’s disease.

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Abstract: Alzheimer’s disease is the most common cause of dementia. The increasing prevalence of the disease presents a challenge to the treating neurologist. Presently the drugs used for Alzheimer’s disease produce only symptomatic improvement. Currently various therapeutic strategies are under development for the treatment and prevention of Alzheimer’s disease. Like γ-secretase inhibitors, immunotherapy with anti Aβ antibodies, Aβ immunization, metal protein attenuation compounds, and peroxysomal proliferators activated receptors γ agonist, NSAAIDS. Alzheimer’s disease has a marked inflammatory component. It is proposed that anti inflammatory therapies may be of value in Alzheimer’s disease. PPARs are members of nuclear hormone receptor super families that are activated by lipids in regulating inflammatory responses. PPARγ has been investigated in animal model for its role due to its anti inflammatory action in various C.N.S. diseases, having an inflammatory component. PPARγ agonists have been demonstrated to suppress amyloid β mediated activation of microglia in vitro and to prevent cortical and hippocampal neuronal death. Various studies have shown that PPAR γ agonists may be of value in treatment of Alzheimer’s disease in animal models. The details of PPAR γ agonists as a therapeutic target for the treatment of Alzheimer’s disease will be discussed.

Abbreviations: PPARs (Peroxysomal Proliferators Receptor Activator Gamma), Aβ (Amyloid beta), NSAAIDS (Nonsteroidal Anti-Inflammatory Drugs), C.N.S. (Central Nervous System).

Experimental studies on antimicrobial potentialities of antipsychotics and antiinflammatory drugs with special reference to their action on Mycobacterium tuberculosis

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Background: Excessive and inadvertent use of antibiotics and antibacterial chemotherapeutics has caused a significant increase in the occurrence of drug resistant pathogens including M. tuberculosis. Systematic search among various pharmacological categories of drugs have revealed that many of them possess potent antimicrobial property.

Methods: The antipsychotics and antiinflammatory drugs were tested in vitro against 300 to 550 bacteria including two cag positive H. pylori and 45 mycobacteria. The MIC was determined by agar dilution technique following NCCLS guidelines along with detection of bactericidal/bacteriostatic action. Intensive in vivo experiments were carried out in mice employing S. enteritis var typhimurium and M. tuberculosis H37Rv. Protection against V. cholerae was established in rabbit ileal loop model.

Results: Antipsychotic drugs fluphenazine, flupenthixol, trifluoperazine and antiinflammatory agent dicitofenac sodium possess remarkable antimicrobial effect against clinical isolates of Gram positive and Gram negative types. Dicitofenac was found to inhibit synthesis of bacterial DNA. These compounds when injected intraperitoneally significantly protected Swiss white mice from the lethality of Salmonella infection. In vitro experiments were performed in mice employing S. enteritis var typhimurium and M. tuberculosis H37Rv. Protection against V. cholerae was established in rabbit ileal loop model.

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Conclusion: Definite detection of antibiotic particularly antitubercular functions in antipsychotics and antiinflammatory drugs may prove to have a profound impact in the treatment of various infections including tuberculosis. In course of time it will be possible to create a new generation of potential antimicrobics by suitable structural modification of these agents. Thus development of novel chemotherapeutic compounds would retain a paramount legacy in the history of therapy of infections as envisaged by Paul Ehrlich.
Targeting of blood vessels by small ApoE-like peptides

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Background: The prerequisite for the successful application of all biological agents is the access to the site of action. Drug application to the brain is a particular challenge. One approach to overcome the blood brain barrier (BBB)-related limitations is the development of drug carriers and their modification with peptides to take advantage of physiology-based strategies. We introduced a modified apoE-derived sequence, Az2 (LRLKLRKLRLL), as target recognizing and uptake mediating compound. The peptide exhibits characteristics of cell penetrating peptides (CPPs), comprises specific binding sites for the low density lipoprotein receptor (LDLR) and binds non-specifically to cell-surface heparan sulfate proteoglycans (HSPGs). These properties provide the potential for the activation of different cellular uptake modes. Introduction of two palmitoyl chains conferred detergent-like properties upon the cationic sequence. This lipopeptide allowed rapid and easy formation of different particulate systems.

Methods: Spectroscopic and calorimetric methods were used to characterise the particles and confocal laser scanning microscopy (CLSM) and fluorescence assisted cell sorting (FACS) were used to monitor cellular uptake.

Results: The peptide mediated efficient non-selective cellular uptake of liposomes into different cell lines such as endothelial cells of brain capillaries and large vessels. The uptake mode into capillary endothelial cells is endocytotic, but neither clathrin nor caveolae mediated. The LDLR does not play a role. Cell surface HSPGs are involved in the uptake process, providing an explanation for non-specificity and leading to the suggestion that electrostatic interactions between the carrier and the LDLR play an essential role in the internalisation process. The uptake of micelles is cell specific. P2A2 micelles are efficiently internalized into capillary endothelial cells whereas uptake into endothelial cells of large vessels is low. The observation implies that on the various cell species different transport routes are activated and the properties of the particulate carrier, such as the size, surface density of the vector peptide, and peptide conformation influence the process.

Conclusion: Our studies lead to the conclusion that small micellar particulate structures with a high surface density of the cationic apoE vector peptide are highly favourable for the uptake into brain capillary endothelial cells.

New neutralizing monoclonal antibodies from HIV-1 subtype C and CRF02_AG infected people.

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Background: The aim is to develop prophylactic human immunodeficiency virus vaccines targeting subtype C and circulating recombinant form CRF02_AG which are currently most prevalent. As potential candidates, a genetic immunization strategy has protected rhesus macaques against infection with a neutralization sensitive SHIV which, however, was constructed from a subtype B isolate albeit a variant of the immunogens. Protection correlated with activity in extended incubation phase neutralization assays against virus prepared in primary human cells. To develop this immunization strategy further, combinations of immunogens which can induce antibodies able to cross-neutralize fully heterogeneous isolates have to be identified. First, monoclonal antibodies with these properties need to be isolated. They can then be used to select recombinant glycoproteins and construct peptides.

Methods: The subtypes of HIV-1 from infected patients attending the clinic in Antwerp were determined. Plasma from individuals infected with subtype C or CRF02_AG was mixed with dilutions of primary virus from a panel of HIV-1 isolates and incubated for 24 hours. Residual infectious virus was quantified following exposure of this mixture to phytohaemagglutinin transformed human peripheral blood mononuclear cells for one hour. The presence of infectious virus was determined by HIV-antigen ELISA of supernatants after 14 days' culture. Reductions in infectious titre were calculated and expressed as neutralization indices. Memory B-lymphocytes from individuals with cross-neutralizing antibodies were isolated, stimulated to divide and immortalized by Epstein-Barr virus. Dividing cultures were initially screened for antibodies binding to recombinant HIV-1 envelope gp140. Neutralizing activity of the monoclonal antibodies was subsequently determined in a standardized pseudovirus HOS.CD4-CCR5 based cell assay.

Results: Patients with plasma showing subtype-associated cross-neutralization were identified. So far, two antibodies with broad cross-neutralizing activity and a potency comparable to currently available human monoclonals have been isolated. Other antibodies have a more restricted range while recognising a variety of known epitopes on the external envelope glycoproteins although a subgroup bound to potentially novel neutralization epitopes.

Conclusions: New cross-neutralizing antibodies are available which can identify immunogens for use in recombinant prime, polyvalent boost immunization strategies against HIV-1 subtype C and CRF02_AG isolates.
Effect of antineoplastic agents on the surface properties of bacterial cells

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Background: The study of the effects of antineoplastic agents on bacteria is important to understand post-chemotherapy infections, generally only ascribed to antibiotics present.

Methods: Exponentially growing cells of Staphylococcus aureus, Rhodococcus erythropolis, Mycobacterium aurum and Escherichia coli were placed in medium containing 100, 50, 25, 12.5, 6.3, 3.1, 1.6 and 0.8 µg/mL of mitomycin C (MC) or cyclophosphamide (CP). The fatty acid composition of the cellular membranes was analysed by gas chromatography. Cell viability and membrane potential were determined by fluorescence microscopy and image analysis, using fluorescent dyes. Culturable cells were determined by the spread plate technique. Cell hydrophobicity was measured by the "microbial adhesion to hydrocarbon" test.

Results: Both MC and CP affected the fatty acid composition of the cellular membrane of all strains. A dose dependent increase in the degree of saturation of fatty acids occurred after exposure to MC. Both agents caused a dose dependent decrease in cell viability, part of the population presenting depolarised membranes. Cells exposed to MC produced biosurfactants, decreasing the medium surface tension and R. erythropolis cells produced significant amounts of exopolymeric substances on agar plates after 24h exposure to both agents. Cell clustering was promoted by increasing concentrations of these drugs. Cells that were able to grow after 24h exposure to each of the antineoplastic compounds, were also able to grow in the presence of 25 µg/mL of chloramphenicol, suggesting cross-resistance between them.

Conclusions: 1) Bacterial cells are able to survive relatively high concentrations of both MC and CP. 2) These cells can also grow in the presence of chloramphenicol. 3) Post-chemotherapy infections could be promoted by an adapted bacterial population after antineoplastic exposure.

The High Cytotoxicity of Cisplatin Nanocapsules in Ovarian Carcinoma Cells Depends on Uptake by Caveola-Mediated Endocytosis

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Background: Cisplatin is one of the most widely used agents in the treatment of solid tumors. However, its clinical use is limited by toxic side effects and by the occurrence of resistant tumor cell sub-populations. We have developed a novel method for the efficient encapsulation of cisplatin in a lipid formulation that may reduce these side effects and increase the therapeutic efficacy of cisplatin in the clinic (Burger et al., Nat. Med. 8, 2002). The method is unique in that it generates nanocapsules, nanoparticles of cisplatin covered by a single lipid bilayer. The nanocapsules exhibit an in vitro cytotoxicity up to 100-fold higher than the free drug toward some but not all human ovarian carcinoma cells. Here we report on the mechanism underlying the cell line dependence of the increased cytotoxicity.

Methods: Cellular platinum accumulation and platinum-DNA adduct formation were analyzed by non-flame atomic absorption spectroscopy. The interaction of fluorescently labeled cisplatin nanocapsules with ovarian tumor cells was investigated by confocal fluorescence microscopy. Endocytic pathways were down-regulated using siRNAs.

Results: The increased cytotoxicity of cisplatin nanocapsules was shown to result from enhanced cellular uptake of encapsulated cisplatin as compared to the free drug, leading to increased formation of platinum-DNA adducts. The origin of the increased accumulation of cisplatin from nanocapsules in the cells was investigated by confocal fluorescence microscopy using nanocapsules containing a fluorescent cisplatin derivative. The results showed that intact nanocapsules are taken up by an energy-dependent mechanism. Co-localization of the fluorescein isothiocyanate labeled nanocapsules with markers of early and late endosomes indicated uptake via endocytosis. Transfection of siRNAs against clathrin heavy chain and caveolin-1 in cell lines that differ in sensitivity to cisplatin nanocapsules, revealed that the increased cytotoxicity only occurs after caveolin-1 mediated endocytosis.

Conclusion: The high cytotoxicity of cisplatin nanocapsules in human ovarian carcinoma cells strictly depends on uptake by caveolin-1 mediated endocytosis.

Pneumocystis jiroveci Dihydroprotease Synthase Gene Mutations and Sulfur Resistance

de la HORDA C 1,2 MORILLA R 1,2 RIVERO L 1,2 FRIAZA V 1,2 GUTIERREZ S 1,2 RESPALDIZA N 1,2 MARTIN-JUAN J 1,3 VARELA JM 1,2

Background: The study of the effects of antineoplastic agents on bacteria is important to understand post-chemotherapy infections, generally only ascribed to antibiotics present.

Methods: Exponentially growing cells of Staphylococcus aureus, Rhodococcus erythropolis, Mycobacterium aurum and Escherichia coli were placed in medium containing 100, 50, 25, 12.5, 6.3, 3.1, 1.6 and 0.8 µg/mL of mitomycin C (MC) or cyclophosphamide (CP). The fatty acid composition of the cellular membranes was analysed by gas chromatography. Cell viability and membrane potential were determined by fluorescence microscopy and image analysis, using fluorescent dyes. Culturable cells were determined by the spread plate technique. Cell hydrophobicity was measured by the "microbial adhesion to hydrocarbon" test.

Results: Both MC and CP affected the fatty acid composition of the cellular membrane of all strains. A dose dependent increase in the degree of saturation of fatty acids occurred after exposure to MC. Both agents caused a dose dependent decrease in cell viability, part of the population presenting depolarised membranes. Cells exposed to MC produced biosurfactants, decreasing the medium surface tension and R. erythropolis cells produced significant amounts of exopolymeric substances on agar plates after 24h exposure to both agents. Cell clustering was promoted by increasing concentrations of these drugs. Cells that were able to grow after 24h exposure to each of the antineoplastic compounds, were also able to grow in the presence of 25 µg/mL of chloramphenicol, suggesting cross-resistance between them.

Conclusions: 1) Bacterial cells are able to survive relatively high concentrations of both MC and CP. 2) These cells can also grow in the presence of chloramphenicol. 3) Post-chemotherapy infections could be promoted by an adapted bacterial population after antineoplastic exposure.

A Simple Microwave-assisted Synthesis of Sulfonamides directly from Sulfonic Acids

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Background: Sulfonamides are an important class of pharmacological compounds with a wide spectrum of biological activities. Sulfonamides have broad applications in many areas of clinical medicine, as excellent, diuretics, anticonvulsants, hypoglycemics HIV protease inhibitors, carbonic anhydrase, and capsaicin inhibitors and in particular as antibacterials.

Methods: We wish to report here an easy and convenient technique for the preparation of sulfonamides directly from sulfonic acid or its sodium salt, improved by microwave irradiation. The method consists of the addition of 1 equiv. of TCT to a mixture of 1 equiv. of sulfonic acid and 1 equiv. of triethylamine in acetone. The method is based on the reaction between the sulfonic acid, which is converted into a sulfonyl chloride, and the amine. The target product is obtained in pure form and in practically quantitative yield, just by concentration of the DCM extracts at reduced pressure.

Results: The analysis showed a 99.7% yield of DCM extracts at reduced pressure. The target product is obtained in pure form and in practically quantitative yield, just by concentration of the DCM extracts at reduced pressure.

Conclusions: A selection of sulfonamides were synthesized from an array of sulfonic acids and the yields were satisfactory in all cases. The methodology is proficient and successful with aromatic and aliphatic sulfonic acids. The reaction is not limited to primary and secondary amines, but works well with hydrazines (entry 3) and amino acids derivatives (entry 4). Anilines are applicable in the reaction (entry 1).
In Vitro Fungicidal Properties of the Plant Saponin, CAY-1, with (1) Two CAY-1 Structurally Related Saponins and (2) Synergism with Silver.

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Background: CAY-1, 1081 and 919 are structurally similar saponins in Capsicum frutescens fruit. CAY-1 is fungidal or inhibitory for many fungi including Aspergillus, Fusarium, Microsporum and Trichophyton species. In contrast, 1081 and 919 are weakly antifungal and inactive, respectively. Historically, silver has been used as an antimicrobial. This study determined the (1) fungidical properties of CAY-1 alone and combined with 1081 and 919 and (2a) fungidical properties of silver and CAY-1 both alone and mixed and (2b) synergy of silver and CAY-1.

Methods: (1) The fungidical activity of CAY-1, 1081 and 919 mixed in ratios 8:1:1, 6:2:2 and 4:3:3 (found in Capsicum), were compared to equal weights of CAY-1 alone. Nongerminated (NG) and germinating (G) conidia of A. flavus, A. niger, A. fumi gatus, F. solani, F. oxysporum and F. moniliforme were tested to determine activity against such conidial types. Separate bioassays (3) were performed (n=12) for each species and conidial type with results analyzed statistically using SigmaStat. (2) This fungidical protocol and Minimum Inhibitory Concentration (MIC) bioassays were performed with dissolved silver and CAY-1 both alone and mixed.

Results: (1) Overall, mixture 4:3:3 was the most active. For A. flavus and A. niger G conidia, CAY-1 alone was significantly (p < 0.001) lethal at 5.3 and 6.6 µg/mL, respectively, and for mixture ratio 4:3:3 at 5.0 and 6.1 µg/mL, respectively. Significant lethality for F. solani G conidia was achieved with CAY-1 alone and 4:3:3 mixture at 19.8 and 6.6 µg/mL, respectively. CAY-1 was inactive against F. moniliforme but mixture 4:3:3 was lethal at 3.0 µg/mL. (2) CAY-1 significantly reduced the G conidial viability of A. flavus, A. niger and F. solani at 1.3, 0.64 and 12.4 µg/mL, respectively. Silver (0.64-79.4 µg/mL) was significantly lethal for all fungal G conidia and the NG conidia of F. oxysporum and F. solani. Combined, silver and CAY-1 had a two times greater concentration effect when tested separately. MIC data showed combined CAY-1 and silver had an additive synergistic effect.

Conclusions: Results suggest that the amount of CAY-1 needed for significant antifungal activity is reduced up to 60% by addition of inactive levels of related saponins or dissolved silver.

The Pharmacokinetics and Pharmacodynamics of Miltefosine for Leishmaniasis.

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Background: Miltefosine is an alkylphosphocholine with activity against leishmaniasis. Pharmacokinetics and -dynamics have little been studied.

Methods: The pharmacokinetics of miltefosine, administered orally to healthy subjects and patients with cutaneous leishmaniasis, is characterised by a rapid absorption and a very slow, two compartment elimination process with elimination half lives of 7 and 31 days. After weeks of oral treatment, miltefosine remains detectable in plasma for up to 5 or 6 months. The effects of hepatic leishmaniasis on drug disposition have not been studied yet.

Toxicity of miltefosine is determined by dose related gastrointestinal adverse effects that are amenable by concurrent food intake and disappear soon after discontinuation of drug administration. The relation between miltefosine plasma concentrations and other adverse effects has not been fully investigated.

The pharmacodynamics of antileishmanial activity of miltefosine is poorly documented. In vitro data show different sensitivity of Leishmania species but it is difficult to extrapolate this to clinical efficacy. Efficacy is usually expressed as cure rates in clinical trials which may be higher than 95% for Indian visceral leishmaniasis but lower for other Leishmania infections. The few available data on parasite clearance indicate that this continues until after discontinuation of miltefosine administration. The pharmacokinetics and -dynamics of systemic treatment for cutaneous leishmaniasis and of topical treatment have not been studied. The little available data on eradication time, parasite sensitivity, host immunity, other pharmacodynamic determinants and the sparse data on drug interactions will be reviewed.

Conclusions: The pharmacokinetics and -dynamics of miltefosine need further study to design rational treatment regimens that address (selection of) resistant parasites, cutaneous leishmaniasis and combination with other antileishmanial agents and to study options for topical therapy.

From immunity theory to anti-infectious chemotherapy. Why awarding two Nobel prices in 1908 to Paul Ehrlich (from Germany) and Elie Metchnikoff (from France). What consequences for medical research?

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By 1908 Elie Metchnikoff and Paul Ehrlich jointly obtained the Nobel Prize of physiology and medicine for their work on immunity. Metchnikoff explained the phenomenon of the phagocytes in observing the starfish larvae (1880), whereas Ehrlich developed the theory of fixing antigen/antibody. The end of the XIX century is dominated by the work of Pasteur and their original model then. He considers that the antigen/antibody reaction is a chemical event which leads Ehrlich to the development of the salvarsan or 606. The therapeutic of the infectious disease get in then the era of chemotherapy. Work on immunity thus were understood to develop the mechanisms brought into play and to generalize the immunological methods with the unit of the infectious diseases. Unfortunately, this very tempting ideal model is put in failure in the case of many infectious diseases and parasitic (tuberculosis, malaria, trypanosomiasis… ). Ehrlich proposes another original model then. He considers that the antigen/antibody reaction is a chemical reaction. So, to fight against a microbe why not introduce into the host, an external chemical body which, like magic bullet neutralizes the cellular target? He carries out its first experiments with a dye: the methylene blue then with the trypanoth, whereas at the Pasteur institute of Paris Roux, Metchnikoff, Mesnil, Nicolle, and Laveran were also working on various dyes (trypanoth, blue trycan, afdrol… ). As the dyes fix on the micro-organisms, they immobilize or kill them. 1903 is a very important year because H. Wolfstein Thomas (English man) proposes to use an arsenical derivative, Atovx, to fight against the African trypanosomiasis. By 1905 he publishes his results in the British Medical Journal. The therapeutic of the infectious disease get in then the era of chemotherapy. Discovered in 1863 by Antoine Béchamp (French chemist), Atovx is the molecule which leads Ehrlich to the development of the salvarsan or 606. If one compares the Nobel conference of the two scientists, that of Metchnikoff is in direct connection with phagocytosis, whereas that of Ehrlich treats already antimalarial treatment, that is to say: chemotherapy. Chemotherapy arised from work on immunity. The intellectual logic of the magic bullet and the cellular target prevailed on the anti-infectious discoveries of chemotherapy and to decree two Nobel Prize in 1908 was thus based a new discipline which was leaded the revolution the XX century: the chemotherapy.
Neuronal trafficking of proteins involved in synaptic plasticity: a GFP-based approach

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Background: Number and distribution of ion channels and receptors involved in synaptic plasticity is modulated by several factors including post-translational control of their intracellular traffic. The improvement of molecular biology techniques and the introduction of GFP-based chimeras has boosted our understanding of the molecular determinants of protein sequences that are relevant for selective protein function. We have combined these molecular biology techniques to fluorescence microscopy and videomaging to analyse the critical steps of assembly, membrane insertion and traffic of small conductance potassium channel type 3 (SK3) and of the p75 neurotrophin receptor.

Methods: Hippocampal cell cultures were transfected with fusion proteins between GFP and different SK3 subunit truncations or P75 GFP-tagged constructs. The distribution of the fluorescent recombinant proteins were analyzed by immunofluorescence confocal microscopy or using a living imaging microscope apparatus.

Results: We analysed full length, truncated versions or mutated constructs of SK3. Qualitative and quantitative image analysis indicated that the full length ion channel distributed in soma, axon and in dendrites, whereas GFP/P75-78-766 (deletion of C-terminal domain), GFP/P75-CARBO (deletion of the calmodulin-binding site) and GFP/PAN (deletion of the N-terminal domain) forms accumulated in the cell body compartment and colocalized with ER marker. The GFP/P75-640-736 form (deletion of the distal C-terminal domain) had a distribution similar to control. The N-terminal deleted construct negatively affected transport and assembly of the full length channel. Movements of intracellular p75GFP were followed by cell imaging at 35°C and found to be sustained by tubulo-vesicular structures acting both anterograde (0.1-0.5μm/s) and retrogradely (0.1-1.1μm/s), with the retrograde transport characterized by two components.

Conclusions: GFP based approach is important for studying molecular and spatial properties of SK3 channel and p75 receptor in CNS neurons. Our data indicate the presence of molecular determinants within the aminoacidic sequence of SK3 protein that are relevant for its intracellular processing; the approach can be extended to the analysis of domains involved in function and pharmacological properties of the channel.

Antithrombetics that do not induce bleeding: the “holy grail” found by interfering with von Willebrand factor

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Background: Platelet accumulation at sites of blood vessel wall damage, e.g. as a consequence of the rupture of an atherosclerotic plaque, is the first step in the formation of an arterial thrombus, cause of amongst others myocardial infarction and stroke. Currently used antiplatelet agents are beneficial in the prevention of such events, however without exception suffer from side effects due to an enhanced bleeding risk.

In arterial thrombosis, adhering platelets to withstand high shear forces induced by the fast flowing blood in stenotic areas, for which they rely on von Willebrand Factor (VWF). VWF is a large multimeric protein present a.o. in plasma, but under normal conditions does not interact with its platelet receptor, glycoprotein Ib. The cryptic GPIbα binding site within VWF becomes exposed, when VWF, bound to collagen exposed in the damaged artery, is stretched by the high shear forces. In particular, the risk of dengue hemorrhagic fever from one of the dengue viruses during a secondary infection appears to rise significantly if there was a previous primary infection from one of the other dengue viruses. This ‘subdominant epitope’ implies that an effective vaccine for dengue must induce protective immunity against all four dengue viruses. To date, no such vaccine has been developed.

Methods: We here explore the possibility of using polytopic, or multi-site, vaccination to induce an effective T cell immune response against all four dengue viruses. Immunization with one dengue virus is protective against future challenge with the immunizing virus. However, immunity built up after infection by one dengue virus protects only modestly or even negatively against reinfection by the other dengue viruses. In particular, the risk of dengue hemorrhagic fever from one of the dengue viruses during a secondary infection appears to rise significantly if there was a previous primary infection from one of the other dengue viruses. This ‘subdominant epitope’ implies that an effective vaccine for dengue must induce protective immunity against all four dengue viruses. To date, no such vaccine has been developed.

Results: We show that specific lysis against the four dengue strains is superior in the multi-site protocol. By physically separating the TCR selection for each epitope in different lymph nodes. We determine whether polytopic vaccination reduces immunodominance and increases recognition of the four dengue viruses.

Conclusions: We here explore the possibility of using polytopic, or multi-site, vaccination to induce an effective T cell immune response against all four dengue viruses. Immunization with one dengue virus is protective against future challenge with the immunizing virus. However, immunity built up after infection by one dengue virus protects only modestly or even negatively against reinfection by the other dengue viruses. In particular, the risk of dengue hemorrhagic fever from one of the dengue viruses during a secondary infection appears to rise significantly if there was a previous primary infection from one of the other dengue viruses. This ‘subdominant epitope’ implies that an effective vaccine for dengue must induce protective immunity against all four dengue viruses. To date, no such vaccine has been developed.

Scultping the Immunological Response to Dengue Fever by Polytopic Vaccination

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Background: The twin challenges of immunodominance and heterologous immunity have hampered discovery of an effective vaccine against all four dengue viruses. Immunization with one dengue virus is protective against future challenge with the immunizing virus. However, immunity built up after infection by one dengue virus protects only modestly or even negatively against reinfection by the other dengue viruses. In particular, the risk of dengue hemorrhagic fever from one of the dengue viruses during a secondary infection appears to rise significantly if there was a previous primary infection from one of the other dengue viruses. This ‘subdominant epitope’ implies that an effective vaccine for dengue must induce protective immunity against all four dengue viruses. To date, no such vaccine has been developed.

Methods: We here explore the possibility of using polytopic, or multi-site, vaccination to induce an effective T cell immune response against all four dengue viruses. We investigate whether injection of the epitopes from each of the four viruses in different physical locations sculpts a broader TCR response, by inducing TCR selection for each epitope in different lymph nodes. We determine whether polytopic vaccination reduces immunodominance and increases recognition of the four dengue viruses.

Results: We show that specific lysis against the four dengue strains is superior in the multi-site protocol. By physically separating the TCR selection for each epitope in different lymph nodes. We determine whether polytopic vaccination reduces immunodominance and increases recognition of the four dengue viruses.

Conclusions: We show that specific lysis against the four dengue strains is superior in the multi-site protocol. By physically separating the TCR selection for each epitope in different lymph nodes. We determine whether polytopic vaccination reduces immunodominance and increases recognition of the four dengue viruses.

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Open Chemical Databases and Ontologies in the Genomic Age

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Modern research in biochemistry and pharmacology depends on availability of chemical data, which until few years ago were almost exclusively concentrated in commercial databases. Not only the scientific community but the human mind as a whole will benefit from open access to chemical data in standard computer-readable format, just as is the case with bioinformatics and genomics. The open access is necessary but not sufficient: for example, patent documents may host a treasure of chemical data, but this treasure is well buried and is not a trivial task to extract even with the cutting-edge text-mining techniques. A number of open-access databases that emerged during the last five years, such as PubChem, ChemSpider and eMolecules, provide free access to millions of compounds. However the quality of data (both structure and annotation) depends on community efforts of chemical data curation. The challenges and achievements in the standardisation of chemical language in biological databases will be presented, with emphasis on three aspects of curation:

1) naming: correct, unambiguous and usable nomenclature
2) drawing: unambiguous, computer- and human-readable 2-D diagrams
3) ontology: linking the entity of interest by defined logical relationships to other entities.

I am going to use the open access chemical databases to illustrate these aspects, with focus on ChEBI, a definitive, freely available dictionary of Chemical Entities of Biological Interest. ChEBI provides standardised descriptions of molecular entities that enables other databases to link to EBI controlled vocabulary annotations to their entries in a consistent fashion.

The challenge (1) is illustrated by cases of conflicting nomenclature systems, such as Preferred IUPAC Names (PINs) and International Nonproprietary Names (INNs). Challenge (2) can be met by software implementation of the IUPAC recommendations of graphical representation of chemical structures. The formalization of chemical ontology remains to be addressed. ChEBI ontology, like other Open Biomedical Ontologies (OBO), is manually built with only limited validation. However, the fundamental difference between chemical ontology and biological ontologies is that the former can be formalised using the features derived from connectivity tables. In order to be usable by wider scientific community, the chemical ontology should be scalable so the new compounds could be automatically assigned the ontological relationships.

Effect of Valeriana officinalis in [3H]Glutamate Binding

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Background: Valerian officinalis root extracts have been used as a sedative and anxiolytic for more than 2,000 years. The most accepted theory establishes that Valeriana extracts interfere with GABA-Aergic neurotransmission. Alternatively, relaxation and sleepiness can be produced if Valeriana reduces the activation of glutamatergic and metabotropic (mGlu) receptors. The objective of our study is to determine the effects of Valeriana extracts preparations on the excitatory neurotransmission through [3H]Glutamate binding to mGlu and iGlu receptors.

Methods: Valeriana extracts were obtained from Pacific Botanicals, Oregon. Valeriana was extracted in ultra pure water (~23ºC) and stirred during 1 hour. Aliquots were centrifuged before being analyzed. Assays were done using synaptic membranes from cerebral cortex from female rats of approximately two months of age. The method used was with [3H]Glutamate (20nM) in a final volume of 500 µL of 50 mM Tris HCl/100 mM KCl buffer, pH 7.4. For the dose response curve, valerian extract concentrations of 4ng/ml – 12mg/ml were used. Non-specific binding was determined in the presence of glutamate 1 mM. All samples were incubated on ice for 40 minutes. The assay was stopped by centrifugation for 30 min at 11,000 rpm, then the supernatant is extracted and the pellet washed with 1 mL of ice-cold buffer. After that, the pellets are resuspended and the radioactivity of the samples was determined in a liquid scintillation counter with 1 mL of scintillation cocktail.

Results: Aqueous valerian extract (1x10-3 – 4x10-6 mg/ml) increase [3H]Glutamate binding to a maximum of 60%. At 0.05mg/ml aqueous Valeriana extracts significantly interact with KA (***P<0.05) but not NMDA, AMPA, L-AP4 and quisqualic acid. In contrast, DCG-IV and EGLU markedly decreased, 37% (**P<0.01) and 28% (**P<0.05), respectively, the [3H]Glutamate binding in presence of valerian extract (4x10-6 mg/ml) demonstrating that there is a high selectivity for mGlu receptor interactions.

Conclusions: 1) The present study demonstrated that Valeriana officinalis extracts selectively interact with mGlu receptors. 2) This selective interaction of Valerian with mGlu receptors may represent an alternative explanation for the anxiolytic properties of this plant. 3) The present study demonstrated that various plant extracts preparations selectively interact with mGlu receptors. 4) This selective interaction of Valerian with mGlu receptors may represent an alternative explanation for the anxiolytic properties of this plant.

Inhibition of experimental Sjögren’s syndrome through immunization with Hsp 60kDa and its peptide aa237-460 – predicting treatment efficacy using multi-plex biomarker profiling

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Background: The aim of this study was to investigate a potential immunomodulatory effect of heat-shock protein 60kDa (Hsp60) on spontaneous experimental Sjogren’s syndrome (SS).

Methods: 7-week old non-obese diabetic (NOD) mice were immunized with eubacterial Hsp60 or a Hsp60-derived peptide (amino acid residue aa237-460).

Results: At 21 weeks of age, non-diabetic mice were investigated for salivary gland inflammation, exocrine function and extraglandular disease manifestations. In addition, biomarker profiles comprising 87 analytes in serum and 75 in saliva were analyzed.

Conclusions: Inhibition with Hsp60 and its peptide aa237-460 significantly reduced SS related histopathology compared to NOD controls. In addition, 50% of Hsp60 and 30% of aa237-460 injected mice retained normal exocrine function. Both treatments induced similar changes in biomarker profiles. Notably, circulating IFN-γ-induced protein (IP)-10 and eotaxin decreased significantly as a consequence of the treatment. Anti-mucocar m3 receptor (M3R) IgG1, IL-10 and leptin in contrast discriminated best between the different treatment groups. Successful prevention of hyposalivation was accompanied by quantitative alterations in 36 biomarkers, of which 19 inflammatory mediators decreased to levels comparable to Balb/c. Low secreted vascular endothelial growth factor (VEGF)-A predicted most accurately success ful prevention of hyposalivation. Low salivary granulocyte colony-stimulating factor (G-CSF) was identified as the best predictor of normal secretory function across the strains.

Conclusion: Immunization with Hsp60 and its peptide aa237-460 led to inhibition of SS in NOD mice. Comprehensive analyses revealed specific biomarker signatures capable of predicting treatment group and treatment outcome. Molecularly involved in inflammatory chemotaxis, vasculostimulation and regulatory pathways coincide the differences displayed by the biomarker profiles.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
Synergy between structural stability and DNA-binding controls the antibody production in EPC/DOTAP/DOPA vesicles and DOTAP/DOPA lipoplexes

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Background: The delivery of nucleic acids using cationic lipids as carriers has been a promising area of research since Felgner in 1987 demonstrated its viability in vitro. We present a comparative characterization of physico-chemical properties, in vivo cytotoxicity and in vivo antibody production of surface-bound DNA on EPC/DOTAP/DOPA and DOTAP/DOPA lipoplexes.

Methods: The DOTAP/DOPA (50:50% molar) and EPC/DOTAP/DOPA liposomes were prepared according to the procedure described by Bangham. EPC/DOTAP/DOPA liposomes were frozen, freeze-dried and rehydrated. The complexation with p/νAX/p65 was carried out at a final molar charge ratio (+/-) 10 and final NaCl concentration of 0.9%. Characterizations: average hydrodynamic diameter, zeta potential; plasmid integrity, determination of the molar charge ratio for complete DNA incorporation into the lipid structure, morphology, plasmid accessibility; phase transition, in vitro cytotoxicity. After 15 or 30 days of mouse vaccination, IgG1 and IgG2a production were evaluated.

Results: The EPC inclusion stabilized the DOTAP/DOPA structure, producing higher phase temperature and lower zeta potential despite a close mean hydrodynamic diameter. Similar morphologies were identified in both structures, but a higher fraction of loaded DNA was not electrostatically bound in EPC/DOTAP/DOPA. EPC also induced a striking reduction in cytotoxicity, similar but a higher fraction of loaded DNA was not electrostatically bound in DOTAP/DOPA lipoplexes.

Conclusions: The in vivo antibody production does not correlate with in vitro cytotoxicity, or with structural stability alone. The synergistic effect of structural stability and DNA electrostatic binding on the surface of structures explains the immunological effects, and produced the required condition for DNA delivery.

Magical interactions between cisplatin, fluorouracil and radiation benefit oesophageal cancer patients

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Background: “Chemo-radiation” using bolus cis-platinum, infusional fluorouracil with concurrent radiation has been in use since the early 1980s and has resulted in major improvements in outcome for oesophageal cancer patients.

Methods and Results: This presentation reviews the author’s 20 years’ experience in this area in the context of experience elsewhere in the world. Issues discussed include:-
- differences in pre-clinical and clinical findings
- dose-response relationships for tumour control and for toxicity
- variations in response relating to gender and age
- the possibility of better combinations of the same agents.

Conclusions: Many of the beneficial interactions between these agents remain incompletely understood and therefore still might be considered “magical”. Better combinations of the same agents should be sought before these agents are discarded.

Synthesis of Some New 1,4,3-Thiadiazol-2-ylmethyl-1,2,4-Triazole Derivatives and Investigation of Their Antimicrobial Activities

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The therapeutic effects of 1,2-4-triazoles have been well studied for diverse pathological conditions including inflammation, cancer, pain, tuberculosis and hypertension. In the past decades, the problem of multi-drug resistant microorganisms has reached an alarming level around the world. For the treatment of microbial infections, the synthesis of new anti-infectious compounds has become an urgent need. For this purpose, several compounds that contain a piperazine or morpholine nucleus possessing antimicrobial activity have been synthesized, some which contains an azole ring also. For instance, while Epenozolid, and AZD0363, which are the members of oxazolidinone class antibiotics, consist of morpholine and oxazolidinone rings bearing with each other via a fluorohemianiline linkage, another antibiotic, Linezolid, contains a piperazine ring instead of morpholine. On the other hand, Itraconazol, posaconazole and ketoconazol are those that are using for the treatment of fungal infections, contains a piperazine and one or more azole ring in their structures.

In recent years, various antitumor drugs have been developed for the treatment of cancer. Among these, some 1,4-triazole derivatives incorporating Cytarabine structure were synthesized as antitumor agents in our laboratory. However, cancer is still a major health problem because of the insufficiency of the conventional methods. Small and linear molecules are suitable for heterocyclic ring syntheses. In this study, some 1,4-triazole derivatives were synthesized from the reactions of sulfonyldihydrazine (1) and screened for their antibacterial activities. All the newly synthesized compounds displayed IR, H NMR, 13C NMR, mass spectral data and elemental analysis consistent with their structures.

Malaria-induced up- and down-regulation of Cytochrome P450: Implications for Pharmacotherapy

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Background: During the last three decades, experimental and clinical studies have shown that infections and inflammatory conditions down-modulate expression and activity of cytochrome P450 enzymes (CYP). A few studies suggested that drug metabolism is depressed in malaria as well. This study was undertaken to extend these observations by investigating the effects of malaria on different CYP isoforms.

Methods: Female adult DBA-2 (D2, N=16) and C57BL/6 (BL6, N=19) mice were infected (I) with Plasmodium berghei (ANKA) and their parasitemia rates (P) determined. An equal number of non-infected mice was matched for age and sex was the control group (C). Monoxgenase activities (CYP1A1: ethoxyresorufin-O-deethylase, EROD; CYP2B: benzyloxysresorufin-O-debenzylation, BROD and CYP2A5: coumarin 7-hydroxylation, COH) were determined in liver microsomes in mice with P higher than 30% (D2) or 20% (BL6). CYP1A apoprotein levels were examined by immunoblotting with an anti-CYP1A antibody. Genotoxic effects (micronuclei in bone marrow cells - BMC) of cyclophosphamide (CPA, 25 mg/kg body wt, CYP2B and 3A- activated), dimethylbenzanthracene (DMBA, 50 mg/kg bw, CYP1A-activated) and ethylbenzene(sulfonate) (EMS, 150 mg/kg fed acting clastogen) were also investigated in BL6 mice. BMC were harvested 24 h (EMS and CPA) or 48 h (DMBA) after treatment.

Results: Results (means±SE; ANDWA Durvet; *p<0.05, 1 x C) were as follows: D2: EROD: 60±4.6* x 92±12; BROD: 52±5* x 85±11; COH: 11±8.5* x 7±9±1.7. Immunoblotting showed that levels of CYP1A protein in liver microsomes of infected mice were lower than levels in controls. Data also indicated that malaria attenuated effects of CPA and DMBA and enhanced that of EMS.

Conclusions: 1) Malaria up-modulates CYP2A5 and down-modulates CYP1A and 2A in the liver. 2) Effects of alkylating agents activated by CYP1A, 2B and 3A were depressed whereas that of a direct-acting agent was enhanced. Taken together these findings suggested that malaria up and down regulates CYP and that it may either increase or decrease effects of drugs depending on the CYP isoforms involved in the activation and or clearance of the compound.
Synthesis, Anti-rhinovirus Activity and Mechanism of Action of New Chromone and Chroman Derivatives

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Background: The human rhinoviruses (HRV) are important pathogens causing most of the upper respiratory tract infections in humans. Although these infections are often mild and self-limiting, the impact on human productivity and on medical costs is enormous. Since more than one hundred serotypes of HRVs make the development of a vaccine impractical, extensive efforts have been focused on the development of effective antiviral agents for the treatment of HRV infections. However, despite the in vitro activity of several compounds, to date only few drugs have shown efficacy in humans and none have been approved for clinical use.

Several flavanoids and flavonoids studied by us exhibited a broad antipicornavirus spectrum. In continuation of the search for more potent and highly selective analogues, we designed, synthesized and tested new (Z)-3-benzylidenechromans, 3-benzyl-2H-chromenes and 3-benzylchromans related to the most active synthetic 3(2H)-isoflavenes and homoisoflavones previously studied by us.

Methods: In preliminary studies, the cytotoxicity of all the compounds was evaluated by measuring the effect on morphology, viability and growth of HeLa (Ohio) cells. The inhibitory activity on HRV 1B and 14 replication was evaluated in a plaque reduction assay, starting from the maximum non-cytotoxic concentration (MNTC). HRV 1B and 14 were selected as representative serotypes for group B compounds. In contrast, only a modest inhibition of HRV 14 replication was observed up to MNTC. On the basis of the high activity and therapeutic index spectrum. In continuation of the search for more potent and highly selective analogues, we designed, synthesized and tested new (Z)-3-benzylidenechromans, 3-benzyl-2H-chromenes and 3-benzylchromans related to the most active synthetic 3(2H)-isoflavenes and homoisoflavones previously studied by us.

Results: All the compounds tested showed a potent and selective anti-HRV 1B activity within micromolar or submicromolar range (IC50s ranging from 0.11 to 0.62 mM). The low cytotoxicities resulted in high therapeutic indexes for all these compounds. In contrast, only a modest inhibition of HRV 14 replication was observed up to MNTC. On the basis of the high activity and therapeutic index spectrum. In continuation of the search for more potent and highly selective analogues, we designed, synthesized and tested new (Z)-3-benzylidenechromans, 3-benzyl-2H-chromenes and 3-benzylchromans related to the most active synthetic 3(2H)-isoflavenes and homoisoflavones previously studied by us.

Conclusion: The compounds tested showed a potent and selective activity against HRV 1B and 14 replication with high therapeutic indexes for all these compounds. In contrast, only a modest inhibition of HRV 14 replication was observed up to MNTC. On the basis of the high activity and therapeutic index spectrum.

Aspirin Non-responders in Thai Ischemic Stroke/TIA Patients

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Background: Aspirin resistance has been defined as inability of aspirin to protect individuals from cardioembolic events or to induce a platelet function test (PFT) response. Most reported information comes from Western patients with coronary artery disease and aspirin resistance is defined by laboratory criteria. The purpose of the study was to look for aspirin non-responders in Thai patients with presented with acute/subacute ischemic stroke and transient ischemic attacks (TIA).

Methods: We prospectively included acute ischemic stroke/TIA patients who were treated at Thammasat hospital during August, 2006–July, 2007 and had already been on aspirin for at least 1 year. Aspirin resistance was defined as a failure of either aspirin’s antiplatelet effect (Platelet Inhibition by PFT), or a low PFT response (Platelet Inhibition by PFT). We used laboratory criteria. The purpose of the study was to look for patients who did not respond to aspirin.

Results: There were 194 acute/subacute ischemic stroke/TIA patients during the study period. Forty-six patients (23.7%) who had already been on aspirin (aspirin non-responders) while having TIA/TIA, were studied. Eighteen patients were on aspirin 300-325 mg and 28 patients were on 81 mg per day. Most patients have taken aspirin 300-325mg/day as secondary prevention, while half of patients taking aspirin 81 mg/day had diabetes mellitus and took aspirin as primary prevention.

Conclusions: Aspirin non-responders are more common than we previously thought. Future study is required to clarify mechanisms of aspirin non-responders in Thai patients.
**Background:** Janus kinases (JAKs) are protein tyrosine kinases which play a crucial role in controlling many cellular processes; dysregulation of JAK expression and activity leads to different disorders as leukemias and lymphomas, auto-immune diseases, and myeloproliferative neoplasms (MPN). Since 2005, different mutated JAKs were characterized. A unique mutation in JAK2, leading to a V617F substitution, is a major molecular event in >95% of the Polycythemia Vera (PV), 50% of the essential thrombocythemia (ET), and 50% of the primary myelofibrosis (PMF) patients. No specific therapy exists against any of these diseases. Especially for PMF, the development of a specific therapy would be useful since the evolution of this disease is usually unfavorable. ~30% of PV and ET cases evolve towards PMF. All three can evolve towards acute myeloid leukemia. Thus, the JAK2 V617F offers a molecular target for drug discovery.

It remains a significant challenge to develop selective inhibitors for JAK given their homology and potential structural plasticity. Since the wild type (wt) JAK2 is important for red blood cell formation and for the action of several cytokines and hormones, ideally, an inhibitor should target selectively JAK2 V617F and not wt JAK2.

**Methods:** Our strategy involved profiling a collection of 1,380 small-molecule compounds from an high-throughput screening (HTS) program NCI-HIT in a dose-response format against a panel of three JAK dependent cellular assays using growth inhibition scoring and overall inhibition of ATP production. Our hits were subsequently assayed in similar assays, and genetic reporter assay in order to identify better candidates for leads development.

**Results:** Among 2000 selected small molecules, 7% inhibited proliferation of cells driven by JAK2 V617F, but about 5% also inhibited proliferation of the cells driven by JAK2wt and by JAK1 V658F, a constitutively active JAK that harbors the homologous V617F substitution of JAK2. 2% of the compounds showed <5 fold selectivity for the cells expressing JAK2 V617F.

**Conclusions:** All JAK2 V617F potential inhibitors respect Lipinski’s “Rule of five” and the “New Lead-likeness Rule” and may have potential for further development.
Role of the cell envelope in the antibacterial activities of cyclic polypeptides against *Escherichia coli*

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**Background:** The role of membrane permeabilisation and disruption in the mechanism of action of some polymyxin analogues against Gram-negative organisms is contentious. The effects of polymyxin B (PMB) and its analogue polymyxin B nonapeptide (PMBN) on *Escherichia coli* envelope integrity should correlate but previous work by other workers suggest different modes of action.

**Methods and Results:** This work has reassessed the biochemical techniques used before, and shown that in contrast to previous studies, PMBN (a well-characterized antibacterial synergist) readily releases periplasmic proteins and LPS from treated *E. coli* at sub-inhibitory concentrations in normal physiological buffer conditions.

**Conclusions:** We conclude that PMBN when tested with appropriate methodology, closely correlates with the early effects of PMB on the cell envelope of *E. coli* and this study shows that it is now consistent with the accepted interactions of membrane-active agents on Gram-negative cells.

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Percutaneous absorption of crotamiton in man following single and multiple dosing.

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**Purpose:** Crotamiton is a topical drug used in the treatment of scabies and pruritus. We determined its percutaneous absorption following single and multiple dosing in normal skin.

**Methods:** We used in vivo measurement of percutaneous absorption of [14C] crotamiton in a multidose regimen by measuring urinary excretion and liquid scintillation counting in three groups of four healthy volunteers. The Feldmann urinary excretion method was utilized to ascertain percutaneous absorption. Our results showed that tape stripping does not increase percutaneous absorption of crotamiton; upon repeated application.

**Results:**

- Single dose: Crotamiton absorption was 1.7% of the applied dose.
- Multiple dose: Crotamiton absorption increased to 5.9% of the applied dose.

**Conclusions:** Crotamiton is a safe and effective topical agent with predictable percutaneous absorption when applied multiple times, indicating its potential for repeated use.

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Dissociation of Multi-molecular Drug Complexes and Multi-site Binding to 7-TM Receptors: Protection, Delivery and Enhancement of Adrenergic Activation by Ascorbate.

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Using capillary electrophoresis (CE), we developed a new method of measuring molecular dissociation constants. Molecular complexes will dissociate in an electric field. The relative concentrations of ascorbate (Asc), norepinephrine (NE) and an Asc-NE complex were detected using CE in the 0-200 V/cm range. By varying the relative concentrations of ascorbate (Asc) and norepinephrine (NE), a dissociation constant at a constant electric field, the Ke, was determined. Extrapolating multiple log Ke/s to 0 electric field generated the Kc for the complex. The addition of ascorbate to NE solutions activating smooth muscle shifted the NE dose-response curve to the left by 0.5 log units, indicating enhancement of NE activity by ascorbate. Since binding of NE by Asc would decrease the free NE concentration, the Asc must dissociate from NE prior to binding. The electric field generated by the cell membrane will exceed 200 V/cm for 8-9 nm from the membrane, spanning nearly the entire space between adjacent cells, thus causing dissociation of Asc-NE complexes in the interstitial fluid. Protein receptors create an electronic shadow protecting the agonist binding site from electric field dissociation. Asc binds to adrenergic receptors in the IM range, binding to a site between the first and second extracellular loops and trapping the adrenergic molecule in its active site, thus effecting the enhancement. This process enhances both alpha and beta 2 adrenergic receptors, increasing contractions of blood vessels and the relaxation of bronchioles, and has been shown to effectively enhance treatment for asthma conditions in horses and sheep. Similar results have been found for Asc enhancement of histamine smooth muscle activation. This finding that both molecules in a molecular complex can both bind to a receptor and manifest that receptor’s activity may be a general phenomenon. Membrane electric fields will dissociate any non-covalent complexes as they approach the membrane.

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Superoxide Dismutase Activity in the Kidney of Mice Exposed to Acute Cadmium Intoxication: Protective Effect of Magnesium Pretreatment

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**Background:** Cadmium (Cd) is not a Fenton metal, but it can indirectly increase production of reactive oxygen species. Literature data indicate that short-term exposure to cadmium can decrease the activities of antioxidant enzymes, one of them being superoxide dismutase (SOD), which contains copper (Cu), and zinc (Zn) in its active site and catalyses the conversion of superoxide anion radical to molecular oxygen and hydrogen peroxide. On the other hand, investigations indicate that excessive intake of bioelements, particularly magnesium (Mg), may antagonize cadmium effects. The objective of the study was to determine the effect of increased oral magnesium pretreatment on superoxide dismutase activity in kidney of mice exposed to acute cadmium intoxication.

**Methods:** Swiss albino male mice were divided into three groups: I - control group (not treated animals); II - Cd group: animals given single oral dose of 20 mg Cd/kg b.w. as aqueous solution of CdO2; III - Mg+Cd group: mice given orally 40 mg/kg b.w. as aqueous solution of Mg(CH2COO)2, 1 h before Cd treatment. The animals were sacrificed by decapitation at 4, 6, 12, 24 and 48 h and superoxide dismutase activity was determined by method of Misra and Fridovich. Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by the LSD post hoc multiple range test.

**Results:** The obtained results show that acute Cd intoxication induced significantly decreased SOD activity in kidney after 6 (P<0.001), 12 (P<0.005) and 24 (P<0.001) h. In the kidney of mice pretreated with Mg, SOD activities were not altered if compared with control group.

**Conclusion:** These results imply a positive role of Mg pretreatment on renal SOD activity in the kidney of mice exposed to acute cadmium intoxication. This could be explained by the fact that under the same experimental conditions Mg had beneficial effect on Cu and Zn kidney content, as we confirmed in our recent investigations: Cd induced decrease of Cu and Zn (which are necessary for SOD function), while Mg pretreatment increased their levels.
Conclusions: The received data support the need for defining microbiological breakpoints to distinguish between the native population and the resistant suppopulation within each bifidobacterial species. Strains antibiotic resistant to clindamycin, erythromycin and tetracycline were identified. The PCR-based screening results indicate the presence of erm(A), tet(M) and van(A) genes. The disc diffusion method showed a good correlation between the two methods.

Results: The results demonstrated that pre-apoptotic activity of synthetic glucocorticoid – dexamethasone induced a apoptosis but L4 and adult worms antigens inhibited apoptosis. 4) The understanding of mechanisms operated during nematode infection is important to predict the immune reactivity not only to other pathogenic factors but also for treatment approach.

Aims: 1) To estimate dynamic of cell apoptosis in different phases of nematode infection in mice infected with H. polygyrus and C.LG5. 2) To estimate if pro-apoptotic activity of synthetic glucocorticoid – dexamethasone (DEX) is neutralized by H. polygyrus antigens. Methods: This study included make mice of three strains: middle fast responder BALB/c, fast responder mice FVB and slow responder mice C57Bl6 infected with H. polygyrus. The intensity of cell proliferation, and cytokine production induced by nematode antige was determined 3, 6, 12, 24 and 30 days post infection Apoptosis of mesenteric lymph node cells (MLNc) ex vivo and in vitro culture was evaluated by FACS.

Results: During ongoing infection the percentage of apoptotic cells including CD4+ was changed and different in evaluated strains of mice (P<0.0001). In C57Bl6 apoptosis of CD4+ cells significantly increased as the histopathological testing of infected mice. In infected group of mice the percentage of apoptotic cells was increased in all strains of mice. The antigen of infective larvase stage L3 induced apoptosis of MLNc both infected (P<0.05) and uninfected (P=0.05) mice but antigen of the next stage- L4 and adult stage inhibited apoptosis in in vitro culture. Adult stages excretory-secretory antigen partially reduced total and CD4+ MLNc apoptosis of uninfected (P<0.001) and infected (P=0.001) mice provided by DEX.

Conclusions: 1) H. polygyrus antigens evoked different level of inflammatory reaction in slow and fast responder mice; 2) in C57Bl6 mice a weak inflammation appeared with accordance of accelerated CD4+ cell apoptosis,3) L3 antigen induced apoptosis but L4 and adult worms antigens inhibited apoptosis. 4) The glucocorticoid pro-apoptotic activity was neutralized by H. polygyrus metabolic extracts.
The Role of ABC Transporters in the Pharmacokinetics of Miltefosine for Leishmaniasis

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Background: The ATP-binding cassette (ABC) drug efflux transporters breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp) are involved in multidrug resistant cancer. Recently, it was shown that human ABC transporters are possibly also involved in drug resistance of the Leishmania parasite by modulating the human macrophage host cell. We investigated whether miltefosine, originally an anticaner drug and now an antileishmanial, is a substrate for BCRP or P-gp and review the literature on this topic.

Methods: The influence of BCRP and P-gp on drug transport, accumulation, and efflux of miltefosine were studied directly in cells overexpressing BCRP1 (MDCKII-Bcrp1), and cells overexpressing MDR1a (LLCPK-MDR1a), grown as a monolayer on a permeable surface. Transport of radioactively labeled was evaluated and compared in transwell-experiments, in triplicate. A review will be given, combining data from literature with our own data.

Results: Miltefosine was not a substrate for BCRP in our cell model. On the other hand, miltefosine was a modest substrate for Pgp (MDR1a). The LLCP-MDR1a cells showed twice as much transport of miltefosine from the basolateral to apical compartment, than vice versa (B-A: 7.5% transport, A-B: 3.4%; means after 4h). A hand, milte fosine was a modes t substrate for Pgp (MDR1a). The LLCP-MDR1a cells showed twice as much transport of miltefosine from the basolateral to apical compartment, than vice versa (B-A: 7.5% transport, A-B: 3.4%; means after 4h). A large amount of miltefosine was internalized by the cells. We elaborate on the role of Pgp in the pharmacokinetics and drug resistance concerning miltefosine.

Conclusions: Miltefosine was shown to be a substrate for Pgp, but not for BCRP. Transport was only influenced by overexpression of Pgp (MDR1a) in our transwell experiments. Further studies are warranted to investigate the role of miltefosine in e.g. antimony resistant leishmaniasis.

L-tryptophan as a Research Compound and Therapeutic Agent

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1University of Texas Health Science Center San Antonio, Psychiatry, Division of Alcohol and Drug Addiction, NRC; 2The Cardift School of Health Sciences, University of Wales Institute Cardff (UWIC). L-tryptophan is a dietary amino acid that has been used as a treatment for mood disorders, either alone or in combination with other pharmacological agents, to augment serotonin function. In the research arena, procedures have been used to reduce L-tryptophan availability and consequently deplete serotonin in the brain, to study the relationship between serotonin and accompanying mood states in participants with various psychiatric disorders. Important findings in this area over the last decade have produced dramatically increased interest in L-tryptophan research. The clinical benefits of L-tryptophan indicate that it could play a significant role in augmentation strategies in the treatment of psychiatric disorders, and findings from studies that alter L-tryptophan availability further underscore its importance for altering mood states.

Circumventing the blood-brain-barrier. Soviet attempts during World War 2.

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Background. Following the outbreak of the WW2, Soviet scientists were asked to contribute to the war effort. Lina Stern (LS), the first and only female member of the Academy of Sciences (AoS), saw an opportunity to serve her country, while at the same time assessing, if what she had learned in animal experiments with by-passing of the blood-brain-barrier, applied also to humankind. She had noted that direct injection of drugs into the spaces containing cerebro-spinal fluid led to different, sometimes even opposing effects, compared to those seen after injections into blood, muscle or peritoneum. Thus, intracisternal (ic) injections could lead to marked stimulation of autonomic functions.

Methods. Our research is based on the few articles on human patients LS published during WW2 in British and American medical journals, on Soviet publications aimed at informing the international community about scientific achievements in the USSR, as well as on experimental protocols kept in LS' archives at the AoS.

Results. During the war against Finland (1940) and Germany (1941 and thereafter), soldiers suffering from traumatic shock received ic injections of potassium phosphate solutions and a majority benefited from such a treatment. LS was rewarded with a Stalin Prize (1943), although some members of the medical corps of the army remained sceptical of her approach. Significant results were obtained with ic antitoxin injection to tetanos-affected patients, although the data is difficult to interpret, as these patients also received the antitoxin intramuscularly. In 1945-46, LS obtained small amounts of streptomycin, the antibiotic discovered in 1944 in the United States which was the first ever to be active against tuberculosis. She gave it to paediatricians to allow them to treat, with “her method”, children affected by meningal tuberculosis, which had a very short life expectancy. The “magic bullet” worked, a majority of children survived, however many with hearing deficits, a side effect attributable to the streptomycin.

Coda. Early in 1947, LS was accused of treason and terrorist activity, arrested and detained for nearly 4 years in the abominable Lubjanka prison in Moscow. Whether this was in part due to the unauthorized obtaining (through personal international contacts) and importing of streptomycin into the USSR remains obscure. More important, however, Stalin was turning antisemitic in his last years and the Cold War was looming large. Notice that LS was born into a family of German-speaking, well educated, Latvian Jews. In July 1952, 14 prominent members of the Jewish Antifascist Committee were sentenced: 13 to death, LS to 5 years of internal exile. In 1953, only weeks after Stalin’s demise, she could return to Moscow, where she lived until her death in 1969, at the age of 90.
Molecular guidance systems for nuclear-tipped magic bullets: therapy protocols.

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Background: It is a prerequisite of rhTSH therapy of differentiated thyroid cancers (DTC) that the sodium iodide symporter be activated in order to maximize iodine uptake. For more than 60 years this was achieved by withholding thyroid hormone replacement for several weeks until thyroid stimulating hormone (TSH) rose into the hypothyroid range. This method of patient preparation is associated with significant side effects in many instances and it prolongs patient time away from work and other normal activities.

Methods: First therapeutic uses were in compassionate care settings with patients who could not be safely rendered hypothyroid (Robbins et al). An international prospective, randomized trial was later performed to compare TSH withdrawal with rhTSH administration. The prospective, randomized trial obtained equivalent clinical outcomes in the two arms at 6-8 months post therapy (Paech et al, 2006). Further, dosimetry showed that identical administered doses of rhTSH resulted in a 30% lower whole body radiation dose to those patients who received rhTSH, since euthyroid kidneys maintain function whereas hypothyroidism lowers glomerular filtration rates by 30%. Longer follow up has confirmed that clinical outcomes remain comparable in the prospective study for at least 3 years (Elsie, 2008) and in a separate retrospective cohort to 8.5 years (Rachinsky, 2006). rhTSH administration. In our own centre, we have also considered the cost implications of the two protocols.

Results: In a retrospective cohort of 115 compassionate care patients it was found that about 40% benefited clinically from rhTSH-stimulated radiodine therapy. The prospective, randomized trial obtained equivalent clinical outcomes in the two arms at 6-8 months post therapy (Paech et al, 2006). Further, dosimetry showed that identical administered doses of rhTSH resulted in a 30% lower whole body radiation dose to those patients who received rhTSH, since euthyroid kidneys maintain function whereas hypothyroidism lowers glomerular filtration rates by 30%. Longer follow up has confirmed that clinical outcomes remain comparable in the prospective study for at least 3 years (Elsie, 2008) and in a separate retrospective cohort to 8.5 years (Rachinsky, 2006). rhTSH administration. In our own centre, we have also considered the cost implications of the two protocols.

Conclusions: 1. rhTSH and endogenous TSH are equally effective in preparation of DTC patients for therapy 2. Further, since circulating radiiodine is excreted more quickly, radiation safety issues are also truncated in time compared to the case of TSH given to hypothyroid patients. 3. In many jurisdictions the cost of rhTSH is an issue for resource-limited health care programs. Cost-effectiveness evaluations show that rhTSH utilization is associated with significantly reduced morbidity, less time away from work and that it is cost-effective in radiodine therapy protocols.

 NRF III – 2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich
Nürnberg, October 3-5, 2008

Interactions of liposomal vesicles with bacterial cells and antimicrobial activity of liposomal antibiotics.

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Liposomes have significant effect as antibiotic carriers on improving drug distribution and decreasing a drug's toxic properties. Liposomal drug formulations were developed to increase the bactericidal efficacy of antibiotics by promoting effective interaction between bacteria and liposomes. Various liposomes containing fluoroquinolones and amoxicilloycides demonstrated reductions in minimum inhibitory concentrations (MICs) compared with the free drug against Gram-positive and Gram-negative bacteria. The antimicrobial activity of PC-Chol:DOTAP cationic liposomes containing meropenem, gentamicin and ciprofloxacin were tested in vitro on Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli strains. Ciprofloxacin loaded liposomes exhibited a 2-4 times higher antimicrobial activity compared with the free drug. The bacterial sensitivity to liposomal meropenem was similar as to free antibiotic. The least effective were the liposomes containing gentamicin. The interactions between cationic liposomal formulations (PC:Chol:DOTAP 3:4:3) and examined bacterial cells were tested by fluorescent microscopy. The study was undertaken because different antimicrobial results had been obtained for liposomal antibiotics. The interactions were examined using PI-stained and labelled liposomes. Some of the strains exhibited red-light emission (fusion with vesicles or vesicles surrounding the cell) and some showed negative reaction (no red-light emission). The microscopic studies showed interactions of all Klebsiella, Pseudomonas and E.coli strains with tested liposomal formulations. Significant variation were noticed for Pseudomonas aeruginosa strains. Surprisingly the fusion effect were observed for isolates resistant to liposomal antibiotics. It seems that the efficacy of liposomal drugs strongly depend on both the outer membrane structure of bacterial cell (interactions that may lead to fusion) and mechanism of bacterial drug resistance. It suggests that if the bacterial resistance mechanism is highly effective even direct drug insertion into the bacterial cell does not significantly change the antimicrobial susceptibility to antibiotics.

Mast cells infiltrate the thalamus as part of the CNS nociceptive response

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2High Dose Ascorbic Acid in Burn Resuscitation
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Abstract
Mast cells (MCs) accessing brain parenchyma through the blood-brain barrier in healthy animals are limited to pre-cortical sensory relays, the olfactory bulb and other normal activities.

Over the past 10-15 years, a series of studies in experimental animals and humans have explored the use of high doses of ascorbic acid in reducing fluid requirements and tissue edema associated with burns. Animal studies have been performed in rats, guinea pigs and sheep at doses as high as 640 mg/kg/24 hr in lactated Ringer’s solution. Studies in humans have infused doses of 66 mg/kg. Primary endpoints in all studies have been total fluid infusion and fluid balance. Secondary endpoints have included hemodynamics and antioxidant status.

Results: Studies in experimental animals have reported significant reductions in fluid requirements to achieve equal hemodynamic benefit as long as the vitamin C was infused within 6 hr of the burn injury. Studies in humans reported reduced fluid requirements, less burn wound edema and reduced ventilator days. No overt toxicity was noted in any study.

Conclusions: To date the data suggest that doses up to 66 mg/kg/hr infused for 24 hr to burn injury in humans may be effective in reducing fluid needs and tissue edema, and such doses have produced no overt acute toxicity. As an antioxidant vitamin, ascorbic acid has been investigated as a therapeutic agent in several disease states. This presentation will review evidence to suggest that ascorbic acid can be used as a ‘magic bullet’ as part of early burn resuscitation practices.

All abstracts are listed in alphabetical order of the presenting author.

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Ganciclovir treatment of infants with cytomegalovirus infection and central nervous system involvement.

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Background: Congenital and acquired human cytomegalovirus (HCMV) infection is very frequent and dangerous especially in the central nervous system involvement. So far vaccination against cytomegalovirus is not possible and the search for effective and safe antiviral drug is on. Ganciclovir (GCV) is one of the “oldest” antiviral drugs against HCMV. In The Children’s Memorial Health Institute in the years 2000-2013 treatment of neonates and infants has been used for several years after the endorsement of the Bioethics Committee and the informed consent of the parents. Aim: Estimation of the efficacy and tolerability of ganciclovir in infants with congenital HCMV infection. Methods: 66 infants at the age from 2 to 12 months with detection DNA HCMV in cerebrospinal fluid by qualitative PCR method were treated with intravenous infusions of ganciclovir. The dose of GCV was established individually after the pharmacokinetic examinations. The longest time of treatment was 12 weeks (3 courses with 1 months interval). The analysis of the blood cell count as well as chemistry were regularly performed. The infants had also neuromaging and electroencephalographic examinations, were taken into multispecialistic care and followed-up.

Results: Epileptic seizures, hypotonia, choreoarthritis, sensorinnural hearing loss, central nervous system malformations, calcifications, hepatoplenomegaly, hepatitis, thrombocytopoenia, anemia were clinical symptoms and signs of the cytomegalovirus infection. In all infants, after antiviral treatment cerebrospinal fluid DNA HCMV wasn’t found in control. GCV treatment was particularly effective in chorioretinitis, hepatoplenomegaly and thrombocytopoenia. After combined antiviral and antileptic treatment infants were long term seizure free and even withdrawal of the antiepileptic drugs was possible in 8 cases. Only transient neutropenia was observed in 5 out of 66 infants (7.5%). No other side effects of antiviral treatment were stated during the follow-up (mean 7 years).

Conclusions: 1) Cytomegalovirus neuroinfection treatment with ganciclovir was effective and well tolerated. 2) Side effects of the GCV treatment were mild and transient.

Phenotypization of Cyclosporine A in Stable Renal Transplant Patients

DURICIOVA J, MARSLAKOVA P, KOMZAKOVA I, BROZMANOVA H, GRUNDMANN M

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Background: The immunosuppressive drug cyclosporine A (CsA) shows broad interindividual pharmacokinetic variability due to different intestinal absorption and metabolism. CsA is metabolized extensively in the liver and intestine by the cytochrome P450 3A (CYP3A) to 3 primary metabolites with the AM1 being the predominant. The CYP3A isoenzymes (CYP3A4 and CYP3A5) show function variability between individual patients and infants. The aim of this study was to compare the values of blood CsA levels and its metabolite AM1 during the 12 h interval with the AUC0-12 calculated by means of HPLC with UV detection. Metabolic ratios (MR) of blood concentration CsA/AM1 and the AUC0-12 were calculated. Spearman’s rank correlation test was used.

Results: There were large intra- and interindividual differences in the MR CsA/AM1 at different blood sample times. In only 10 out of 39 patients (28%) the MR CsA/AM1 indicated the same metabolic group throughout the 0-12 h blood sample time interval. However there were significant correlations between MR CsA/AM1 and MR AUC0-12 CsA/AM1 at the particular blood sample time, the strongest correlation was found with the MR CsA/AM1 at 5 h after CsA intake (r=0.9501, P<0.0001). The MR AUC0-12 CsA/AM1 was then divided into 3 metabolic groups with 16 patients having the MR 0.6-1.6) and thus presenting the highest metabolic activity, with 15 patients having MR 1.7-3.1 and 8 patients having the highest MR (3.2-5.3) and presenting the lowest metabolic activity.

Conclusion: Our results show that the MR AUC0-12 CsA/AM1 correlated with all the MR CsA/AM1 throughout the 0-12 h blood sample time interval, however the strongest correlation was found with the MR CsA/AM1 at 5 h after CsA intake.
Current knowledge on membrane transporters of vitamin A and its precursors

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1Université catholique de Louvain, Louvain-la-Neuve, Belgium; 2The Ohio State University, Columbus, USA.

Background: Humans must obtain vitamin A (or retinol (ROL)) from the diet either as preformed ROL or as provitamin A carotenoid precursors to maintain some vital functions. Both deficiency and excess of ROL are known to cause pathologies. Thus, a better understanding of the mechanisms of intestinal vitamin A absorption is important to optimize amounts in the diet. Aims: 1) To define mechanisms of absorption of ROL/carotenoids. 2) To focus on potential membrane transporters.

Methods: In presence of oleate/taurocholate, differentiated Caco-2 cell monolayers on inserts are able to produce chylomicrons (CM). ROL and carotenoids were delivered to cells using Tween 40 (0.1%). Glyburide was used at 0.2 or 1.0 mM. Lipoprotein fractions (CMs and VLDL) in the basolateral medium (BM) were isolated by ultracentrifugation. [3H]-Glycerol was used to label newly formed triglycerides and follow CM. Lipids (including retinoids/carotenoids) in cells and media were extracted by solvents, triglycerides isolated by TLC and [3H] counted, and retinoids and carotenoids analysed by HPLC. Inhibitions of SR-B1, Glucose phosphate in a lower degree (+). and media were counted, and retinoids and carotenoids analysed by HPLC. Inhibitions of SR-B1, Glucose phosphate in a lower degree (+). and media were counted, and retinoids and carotenoids analysed by HPLC. Inhibitions of SR-B1, Glucose phosphate in a lower degree (+). and media were counted, and retinoids and carotenoids analysed by HPLC. Inhibitions of SR-B1, Glucose phosphate in a lower degree (+).

Results: When cells were incubated with ROL for varying times, cellular ROL plateaued within 2h, whereas retinyl ester (RE) formation increased continuously. ROL and RE efflux into BM increased linearly with time. ROL in the non lipoprotein fraction and REs in CM. Lipids (including retinoids/carotenoids) in cells and media were extracted by solvents, triglycerides isolated by TLC and [3H] counted, and retinoids and carotenoids analysed by HPLC. Inhibitions of SR-B1, Glucose phosphate in a lower degree (+). and media were counted, and retinoids and carotenoids analysed by HPLC. Inhibitions of SR-B1, Glucose phosphate in a lower degree (+). and media were counted, and retinoids and carotenoids analysed by HPLC. Inhibitions of SR-B1, Glucose phosphate in a lower degree (+). and media were counted, and retinoids and carotenoids analysed by HPLC. Inhibitions of SR-B1, Glucose phosphate in a lower degree (+).

Conclusions: 1) ROL enters intestinal cells by diffusion. 2) ROL efflux is partly facilitated, probably by the basolateral transporter ABCA1. 3) Newly-synthesized REs, but not preformed esters, are incorporated into CM and secreted. 4) Carotenoid uptake is mediated by the apical transporter SR-B1 and carotenoid efflux occurs exclusively via their secretion in CM.

Sulfated oligosaccharides as main targets in cruzipain, the major cysteine proteinase of Trypanosoma cruzi

ACOSTA DM3, SOPRANO LL1, ESTEVA MI1, KOVENSKY J2, COUTO AS3

DUSCHAK VG1

1Inst Nac Parasitol "Dr Mario Fatah Chaben", Ministerio de Salud, Argentina; Laboratoire des glucides, Université Joules Verne, Amiens, France; 2CHIDECAR, FCEyN, UBA, Argentina.

Background: Trypanosoma cruzi, the agent of Chagas disease contains a major cysteine proteinase, cruzipain (Cz). This lysosomal enzyme bears an unusual C-terminal domain (C-T) that contains post-translational modifications and most antibodies in natural and experimental infections are directed against it.

Methods: To address the structure of the N-linked oligosaccharides present in the C-T domain, UV-MALDI-TOF mass spectrometry was used in conjunction with peptide N-glycosidase F deglycosylation and high performance anion exchange chromatography. In order to evaluate the immune responses to sulfated moieties on Cz, and the involvement of anionic charged structures in the immune recognition of sulfated glycoproteins, BALB/c mice were immunized with purified Cz and C-T prior and after desulfation treatment.

Results: The MALDI-TOF MS analysis allowed us to identify and characterize a new striking feature in cruzipain: sulfated high-mannose type oligosaccharides. The humoral immune response to sulfates on Cz or C-T was mainly IgG2b. IgG2b reactivity was abolished when desulfated antigens were used as immunogens showing that sulfates are absolutely required for eliciting IgG2b response to Cz. A significant reduction of C-T-specific delayed-type hypersensitivity reaction in C-T-immunized mice was observed when desulfated C-T was challenged, suggesting the involvement of sulfate groups in the generation of memory T-cell responses. Moreover, immunization with C-T elicited ultrastructural abnormalities in heart tissue. Surprisingly, hearts from sulfate-depleted C-T-immunized mice did not show pathological alterations. In contrast to anti-desulfated Cz mice serum, anti-Cz serum recognized sulfated poligalacturonic acid with relation So4/COO = 1(++)1.07 (++); 0.4 (+); 0.1 (-) and Glucose phosphate in a lower degree (+).

Conclusions: We show for the first time 1) the presence of sulfated glycoproteins in Trypanosomiasis; 2) that sulfates are able to elicit specific immune responses and appeared to be involved in the generation of heart tissue damage. 3) Our findings suggest that this effect could not be specifically due to sulfates but to anionic charged structures.

Authors’ disclosure statement: Sera from chronically T. cruzi-infected subjects with mild disease displayed higher levels of total IgG and IgG2 antibodies specific for sulfated epitopes compared with those in more severe forms of the disease.
Rheumatoid arthritis, Proteus and “magic bullets”.

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Rheumatoid arthritis (RA) affects over 5 million people in the European community and over 20 million throughout the world. Stastny's discovery in 1976, that RA patients carry HLA-D4 led to the identification of the “shared epitope” (EQR) found in HLA-DR1/4 individuals. Over 80% of RA patients belong to the HLA-DR1/4 groups, whilst the frequency of these genes in the general population is about 35%.

Immunochemical and molecular analysis showed that the sequence ESRRAL, which resembles the “shared epitope”, is found in Proteus haemolyticus whilst another sequence in Proteus urease crossreacts with type XI collagen found in huylne cartilage.

Antibodies to Proteus bacteria have been found in RA patients from 14 different countries and RA sera have cytopathic properties against sheep red cells coated with EQR/KRAA peptides.

It would appear that RA is caused by an upper urinary tract infection by Proteus bacteria and this would explain why this disease occurs more frequently in women than men.

Therefore antibiotics or Ehrlich “magic bullets” against Proteus bacteria should be used as specific therapeutic agents together with non-specific drugs, such as anti-TNF, biologicals, DMARDs and NSAIDs in the treatment of RA.

The assessment of specific anti-Proteus magic bullets in the treatment of RA is long overdue.

Disinfectant effect of Garcinia kola extract on Staph. Aureus

EGWUATU CA*

The methonalic extract was more effective than the aqueous extract with MIC of 30.9 ± 0.2

in-vitro using the agar dilution method. The minimum inhibition (MIC) was evident of growth.

In the general population is about 35%.

An inhibition of 405 nm along all the concentration of study, revealed that the AmB molecules were strongly and individually bound to the ME droplets.

Table 1. Mean particle size of ME and AmB-ME by photon correlation spectroscopy (Light Scattering)

<table>
<thead>
<tr>
<th>Diameter (nm)</th>
<th>Polidispersity index</th>
<th>pH</th>
<th>Refraction index</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME</td>
<td>30.9 ± 2.1</td>
<td>0.218 ± 0.014</td>
<td>6.0 ± 0.0</td>
</tr>
<tr>
<td>AmB-ME</td>
<td>310.9 ± 20.1</td>
<td>0.361 ± 0.020</td>
<td>7.0 ± 0.0</td>
</tr>
</tbody>
</table>

Conclusions: 1) A novel formulation of AmB-ME was prepared by a straightforward and fast procedure. 2) This new formulation presented the same efficacy of the Fungzon against C. albicans and a lower toxicity against human RBCs. 3) Taken together, these results suggested that ME is an eligible drug carrier for AmB or other water insoluble molecules, and it has potential applications.

Prediction of species-specific targets for the development of STAMPS based on analyses of bacterial species-level supragenomes

EHRLICH GD, HOGG JS, JANTO B, AHMED, A, HILLER NL, BOISSY R, POWELL E, YU S, POST JC, HU FZ
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Background: Current antimicrobial strategies will fail as they rely on broad-spectrum antibiotics that target common cellular functions resulting in the selection and dissemination of genetically encoded resistances that are passed, via horizontal gene transfer (HGT) mechanisms, among pathogens and commensal flora.

Methods: To break this chemical arms race we decided to identify species- and strain-specific targets using 454-based whole genome sequencing and a suite of in-house developed comparative genomic tools to analyze dozens of strains of the human pathogens Haemophilus influenzae, Pseudomonas aeruginosa, Escherichia coli, Shigella flexneri, Staphylococcus aureus, and Streptococcus pneumoniae.

In each case we chose strains that were associated with a spectrum of clinical presentations to obtain the most complete species-level supragenome possible.

Results: For all species the core genome (those genes shared among all strains) contained only a minority (20-30%) of the genes present within the species supragenome. Thus, the distributed genome was 2.5 to 4x that of the core genome, and each strain of a species contains a unique set of distributed genes with respect to all other strains. Importantly, each species only carried a small number of genes that were completely unique to that species. On average, for all species examined, the mean number of genic differences for any pair of strains within a species was between 350 & 600 genes, that 20-33% of each strain’s genome is unique as compared to all other strains of the species. Each of the core genes for each species gives a unique phylogeny, proving that HGT is the major mode of evolution and that intraspecific phylogenetic studies are meaningless.

Conclusions: We are using our comparative genomes pipeline together with a suite of annotation and metabolic programs to identify species- and disease phenotype-specific targets for antimicrobial strategies. We will then develop STAMPS (selectively-targeted gregtricidal peptides) directed towards these diagnostic molecular moieties to specifically target particular pathogens without the risk of broad-spectrum targeting that promotes the rise and spread of antimicrobial resistance cassettes.
Use of retinoids as environmental contamination biomarkers in aquatic ecosystems

EL BOUHALI B1, NASRI I2

1National Laboratory of Forensic Science, Casablanca, Morocco; 2Hassan II University, FST, Laboratory of Biochemistry, Mohammedia, Morocco.

Background: Xenobiotics discharged into the aquatic environment are important to monitor. There are several advantages in using biomarkers instead of traditional chemical analysis. Biomarkers measure integrated responses in time and space to evaluate the state of the environment health. To use the rates of retinol (R) and retinyl palmitate (RP) as biochemical markers of stress, and upregulating the endogenous antioxidant machinery, like GSH.

Methods: The sampling of Gambusia holbrooki fish was made in the Fouarat Lake situated in north-east of Morocco and Sebou estuary. At the laboratory (L), a sample was bred and fed under poor retinol diet. Another sample was bred in external basin in natural conditions. The HPLC assays were performed on a Gilson model 307 with using UV/Vis detector at 325 nm. Separation was made using a Nucleosil C-18 column (250 x 4.6 mm). The isoeluion elution was realised with methanol/water (90:10, v/v), flow rate 1.6 ml/min for the (R) separation and 100 % ethanol at a flow rate of 1.5 ml/min for the (RP).

Results: (R) determination in the body informs about plasmatic rate whereas the (RP) quantification informs about hepatic retinoid reserve. The comparison of different concentrations in (RP) indicates that fish of the estuary have the highest significant hepatic reserve in comparison with that from other sites. The laboratory’s sample possesses the lowest hepatic reserve. (R) concentrations of Fouarat Lake and Sebou Estuary are nearly identical and significantly higher than those of (L) and external breed.

Conclusions: 1) RP and the R may be useful as sensitive biomarkers for monitoring chemical contaminants in Gambusia in freshwater and estuarine sites. 2) Use of (RP) and (R) indicate if exposure to chemicals was respectively chronic or acute. 3) A simple, rapid and inexpensive method was developed for extraction and analysis of (R) and (RP) in Gambusia by reversed phase HPLC.

Plasmoidal Plasma Membrane: isolation and its implication in drug transport

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Background: A model to study drug transport across the plasmoidal plasma membrane is important to investigate the mechanism involved in drug accumulation and resistance in the maternal parasite. Aims: 1) To obtain parasite plasma membranes purified from Plasmodium falciparum. 2) To investigate the role of Pgh1 and 3) to study chloroquine accumulation and ATPase activity in the purified plasmoidal plasma membrane.

Methods: To obtain the parasite plasma membranes in the form of vesicles, trophozoites released by saponin treatment from infected-erythrocytes were purified using anti-erythrocyte antibodies fixed to polystyrene beads and biotinylated to facilitate their recovery with a magnetic system prior to disruption by nitrogen cavitation. The effect of chemosensitisers on ATPase activity and chloroquine accumulation capabilities of the plasma membranes were determined. The effect of anti-Pgh1 antibodies on chloroquine accumulation was also investigated. Pgh1 was identified in plasma membranes isolated from various strains of P. falciparum and the subcellular localisation of Pgh1 in infected-erythrocytes was examined.

Results: Subcellular localisation of Pgh1 indicated that this protein is present in P. falciparum plasma membrane but no link between the overexpression of Pgh1 and chloroquine sensitivity of the P. falciparum strains tested could be found. In addition, polyclonal antibodies directed at Pgh1 were unable to inhibit chloroquine accumulation in purified plasma membranes, suggesting that Pgh1 is not involved as a chloroquine transporter in the plasma membrane of P. falciparum. Verapamil and other agents known to reverse chloroquine resistance by increasing chloroquine accumulation in paralysed erythrocytes did not affect either the lethal effects of CP, and showed a significant recovery of renal function; while the extent of tumor progression was highopy reflected by CRP levels. 2) In rats, EGCG, but not RSVL, obliterated the lethal CP-induced nephrotoxicity and renal tissue injury by reducing oxidative stress, and upregulating the endogenous antioxidant machinery, like GSH.

Abstracts
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All abstracts are listed in alphabetical order of the presenting author.
**Process Research on a Key Synthetic Intermediate of Clopidogrel**

Okayama Univ., Okayama, Japan

**Background:** Clopidogrel is a platelet aggregation inhibitor widely administered to atherosclerotic patients with the risk of a heart attack or stroke that are caused by the formation of a clot in the blood. Worldwide sales of Plavix (clopidogrel bisulfate) amounted to $6.4 billion per year, which ranks second. Here we report the environmentally benign and practical chemoenzymatic synthesis of a key intermediate for clopidogrel, (R)-1, in detail.

**Methods:** α-Keto ester 2 was prepared according to the literature, and the whole-cell reduction of 2 was done with the recombinant E. coli. The results are summarized in Table 1.

**Results:** The desired alcohol (R)-1 was obtained at 30 °C in 76% yield with > 99% ee (entry 1). Table 1 outlines how we optimized the productivity by changing the substrate concentration and the reaction temperature. When the reaction temperature was decreased by 5 °C, the conversion and isolated yield increased (entry 2), which prompted us to double the substrate concentration. Even at the substrate concentration of 0.8 M, the conversion reached 94% (entry 3). Therefore, we further increased the substrate concentration up to 1.0 M, which resulted in 90% conversion (entry 4). Finally, we further lowered the reaction temperature (entries 5 and 6) to find the best temperature giving the highest conversion at the same substrate concentration. Thus, the whole-cell reduction of 1.0 M of 2 at 20 °C gave 98% conversion and 1.78 g of isolated product (R)-1 (entry 5), which corresponds to the productivity of 178 g/L (weight of isolated product per liter of initial reaction volume).

**Conclusions:** In summary, the present biotransformation provides an efficient and greener route for the synthesis of the key chiral carbon synthon (R)-1. The hydride source is glucose, which is the cheap biomass, and the catalyst is E. coli, which can be multiplied easily and inexpensively. The reaction is performed in an aqueous solution under air. This is the first example of the direct asymmetric synthesis of (R)-1 with > 99% ee. Excellent productivity as high as 178 g/L has been achieved. Because of the pharmaceutical value of the downstream product, clopidogrel, this bioprocess has good potential for an industrial application.

**Discovery of Malaria Vaccine Candidates – Application of the HT Cell-Free Protein Production System Born of the Study on Ricin Toxin**

Ehime University, Matsuyama, Japan.

**Background:** Selection of expression systems plays a crucial role in the post-genomic studies on the structure and function of proteins. Although a variety of cell-based expression systems have been widely used for a long time, they have inherent limitations in terms of their adaptability to high-throughput screening and production, and the quality of the proteins produced. Many of these limitations can be circumvented by the use of cell-free translation systems. Among them, the wheat germ based system is of special interest for its eukaryotic nature; it has the inherent limitations in terms of their adaptability to high-throughput screening and mass production, and the quality of the proteins produced. Many of these limitations can be circumvented by the use of cell-free translation systems. Among them, the wheat germ based system is of special interest for its eukaryotic nature; it has the advantage of producing eukaryotic multi-domain proteins in a folded state. However, one shortcoming was overcome by the advent of a new method for the preparation of wheat germ extract, that was based on an idea obtained through my study on molecular mechanism of action of ricin toxin which catalytically inactivates ribosomes. Combining other elementary technologies developed, we established the protocol for the practical use of the wheat cell-free system. The technology consists of (1) in-silico selection of suitable genes from Escherichia coli, (2) in vitro selection of temperature for transcription by the split-PCR, and method for (3) transcription and translation reaction. There are two variations of the protocol, one is for genome-wide production of mutants, the other is for massive production of protein, have been successfully incorporated into task-specific robots that permit fully automated transcription, translation, and purification overnight.

**Conclusions:** I introduce how the practical cell-free system is born of the study on ricin toxin which catalytically inactivates ribosomes. Combining other elementary technologies developed, we established the protocol for the practical use of the wheat cell-free system. The technology consists of (1) in-silico selection of suitable genes from Escherichia coli, (2) in vitro selection of temperature for transcription by the split-PCR, and method for (3) transcription and translation reaction. There are two variations of the protocol, one is for genome-wide production of mutants, the other is for massive production of protein, have been successfully incorporated into task-specific robots that permit fully automated transcription, translation, and purification overnight.

**Methods and Results:** Protein Production System Born of the Study on Ricin Toxin

**Results:** The correct use of drugs especially local injection of glucantime by the physi side effects of systemic therapy of glucantime and reduce the cost which is beneficial for the environmentally benign and practical chemoenzymatic synthesis of a key intermediate for clopidogrel, (R)-1.

**Table 1. Asymmetric reduction of 2 with recombinant E. coli**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Yield (%)</th>
<th>ee (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>92</td>
<td>&gt;99</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
<td>&gt;99</td>
</tr>
<tr>
<td>3</td>
<td>84</td>
<td>&gt;99</td>
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<td>4</td>
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<td>5</td>
<td>88</td>
<td>&gt;99</td>
</tr>
<tr>
<td>6</td>
<td>82</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

**Discovery of Malaria Vaccine Candidates – Application of the HT Cell-Free Protein Production System Born of the Study on Ricin Toxin**

ENDO Y

Ehime University, Matsuyama, Japan.

**Background:** Selection of expression systems plays a crucial role in the post-genomic studies on the structure and function of proteins. Although a variety of cell-based expression systems have been widely used for a long time, they have inherent limitations in terms of their adaptability to high-throughput screening and production, and the quality of the proteins produced. Many of these limitations can be circumvented by the use of cell-free translation systems. Among them, the wheat germ based system is of special interest for its eukaryotic nature; it has the significant advantage of producing eukaryotic multi-domain proteins in a folded state. All of those conventional cell-free systems, however, were plagued by their short lives and as a result, inefficient protein production.

**Methods and Results:** This shortcoming was overcome by the advent of a new method for the preparation of wheat germ extract, that was based on an idea obtained through my study on molecular mechanism of action of ricin toxin which catalytically inactivates ribosomes. Combining other elementary technologies developed, we established the protocol for the practical use of the wheat cell-free system. The technology consists of (1) in-silico selection of suitable genes from the database, (2) in vitro selection of temperature for transcription by the split-PCR, and method for (3) transcription and translation reaction. There are two variations of the protocol, one is for genome-wide production of mutants, the other is for massive production of protein, have been successfully incorporated into task-specific robots that permit fully automated transcription, translation, and purification overnight.

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Spider silk proteins – a new generation of biodegradable materials.

ENGSTRÖM W, JOHANSSON J, YADAGAMA P², HATTON P², WAGEMAKER G, SKIER N³, VOLLRAHT P³, LARHED A³, HABIBOVIC P³, VON ALUOCK S³.

SPIDERMAN CONSORTIUM. 1) Swedish University of Agricultural Sciences, 2) Queen Mary University of London, UK, 3) Sheffield University, UK, 4) Erasmus University, Rotterdam, the Netherlands, 5) Oxford Biomaterials, Newbury, UK, 6) University of Oxford, UK, 7) Q-Med Uppsala, Sweden, 8) University of Twente, The Netherlands, 9) University of Konstanz, Germany

Background: Spider silk consists of fibrous proteins containing highly repetitive sequences of amino acids stored in liquid form that configure into fibres when sheared or spun at secretion. This multiparticle project aimed at recombinant production of this elastic tough and tensive polypeptide fibre that can be used for development of implantable surgical devices.

Methods: This study has used a vast range of recombinant technology structural chemical and immunological methodology in combination with a range of in vitro and in vivo approaches.

Results: We have produced strong tensile fibres originating from a partial cDNA sequence from the Euprosthenopha Spidroin i gene with biomechanical properties that allow the fibres to be used for the development of implantable surgical material.

In parallel progress has been made on the development of alternative material originating from silkworm silks. This class of materials can be used as a scaffold for in vitro synthesis of certain artificially produced biological tissues. Moreover, it has been shown that the different silks form scaffolds that support differentiation and growth of mammalian stem cells. Furthermore, biocompatibility parameters of bone fide, reconstituted and recombinant silk will be presented. A novel in vitro system for assessing immunogenicity as measured by release of selected interleukinS from cultivated macrophages has been developed. The characterisation of spider silks ability to release pyrogens and interleukins from target cells has also been deployed. It has been demonstrated that biosynthetic fibers from Euprosthenops and refined silkworm silk fibers are can be virtually devoid of LPS contamination and show a promising performance in vivo.

Conclusion: Novel fibrous protein biomaterials have been developed that possess the required biomechanical properties to proceed into the development of implant prototypes.

Biopharmaceutical and Pharmacokinetic Searches of Drugs in Perinatology (1968-2008)

ERASHVILI V, KINTRAIA P, GOTOSIRDOZE E, KINTRAIA N, ERASHVILI T, DUGASHVILI N, PAPASHVILI G, KVIZHINADZE N

Scientific Center of Biopharmacy, Tbilisi, Georgia.

Background: As it’s known the biopharmaceutical factors influence on the pharmacokinetic parameters of medical drugs and therapeutic effectiveness. Since 60th of m. an age of pharmaceutical industry the biosynthesis of pharmacokinetics parameters of drugs became important. The pharmacokinetics factors parameters of the drugs are very important. The knowledge about these factors allow an understanding the pharmacokinetics parameters of drugs and a possibility to develop the new forms of medical drugs.

Methods: Our studies worked out by the following directions:

1) To implement the sensitive methods of determination of medical drugs in biological fluids and experimental animals’ organs;

2) To implement the sensitive methods of determination of medical drugs in biological fluids and experimental animals’ organs;

3) To select the release the polypeptides and triichokiddoxidic drugs to create the property material; prepare rational drug forms, investigation their pharmacokinetics and biopharmaceutical data.

For the determination of pharmacokinetic parameters and study the biopharmaceutical factors of drugs we used the following compounds: Aminotrim (10), Oxybutirat (20), Ethmoxini (10), Euprosthenops (20), A. euphoroides (10), A. tsangii (10), and A. euphoroides (10). In this study we focused on 18 triterpenoidal compounds from 5 Aglaia species.

Methods: A total of 18 pure triterpenoidal compounds isolated from 5 Aglaia species (A. ignea, A. dupearena, A. cucullata, A. euphoroides and A. tsangii) were screened against the human immunodeficiency virus type 1 (HIV-1), human adenovirus (HAdV) and the respiratory syncytial virus (RSV) using cell lines permissive for these viruses. Toxicity of the compounds to the cell lines was measured in parallel using either the MTT method or a standard ATP-based assay. Mechanistic evaluation of the potent compounds was carried out using time-of-addition studies.

Results: While only one compound (dammarenolic acid) displayed very potent and selective activity against HIV-1 and HSV-1, three compounds (dammarenolic acid, agaiol and niloti cin) displayed selective anti- HSV-1 activity. Time of addition studies showed that dammarenolic acid (DA) and agaiol (AG) targeted both entry and post-entry steps in the viral replication cycle of test virus (against HIV-1 and RSV for DA and AG for AG).

Conclusions: Dammarenolic acid, agaiol and niloti cin represent novel plant-derived compounds from Aglaia that could be further exploited as suitable leads for the development of potent anti-HIV-1, anti-HSV and anti-HSV agents.

Novel Phyto-antiviral Leads from Aglaia Species

ESIMONE CO, ECK G², DUONG TN, OBERLA K², PROKISCH P²

University of Nigeria, Nsukka, Nigeria, ²Heinrich-Heine University, Düsseldorf, Germany, ³Ruhr University, Bochum, Germany, ⁴Vietnamese Academy of Science and Technology, Hanoi, Vietnam.

Background: Medicinal plants have consistently served as suitable lead sources on potent antiviral, antimicrobial and other pharmacological agents. The genus agaiola contains more than 100 species, many of which have been shown to possess diverse pharmacological activity. In our current search for novel plant-derived antiviral compounds effective against HIV and other human pathogenic viruses, we focused on 18 triterpenoidal compounds from 5 Aglaia species.

Methods: A total of 18 pure triterpenoidal compounds isolated from 5 Aglaia species (A. ignea, A. dupearena, A. cucullata, A. euphoroides and A. tsangii) were screened against the human immunodeficiency virus type 1 (HIV-1), human adenovirus (HAdV) and the respiratory syncytial virus (RSV) using cell lines permissive for these viruses. Toxicity of the compounds to the cell lines was measured in parallel using either the MTT method or a standard ATP-based assay. Mechanistic evaluation of the potent compounds was carried out using time-of-addition studies.

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Conclusions: Dammarenolic acid, agaiol and niloti cin represent novel plant-derived compounds from Aglaia that could be further exploited as suitable leads for the development of potent anti-HIV-1, anti-HSV and anti-HSV agents.
Recombinant Viral Vectors as Suitable Surrogates for Antiviral Screening

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Background: For high throughput antiviral screening studies, safe, rapid, reproducible and cheap screening techniques are needed. Reporter gene-based screening assays are increasingly being recognized as suitable probes for such assays. Here we describe the use of recombinant retroviral and adenoviral vectors expressing either the luciferase (Luc) or green fluorescent protein (GFP) reporter genes as suitable surrogates for the efficient high throughput screening of antiviral compounds from plant or synthetic sources.

Methods: About fifty plant-based and two synthetic compounds were screened for anti-HIV activity using recombinant single-cycle infectious lentiviral vectors expressing either the Luc or GFP gene. To increase tropism the vectors were pseudotyped with the vesicular stomatitis virus Glycoprotein env. Infectious lentiviral vector particles generated by transient co-transfection of the vector plasmid with packaging plasmids were incubated with various concentrations of the antiviral compounds and then used to transduce HeLa cells in 96-well plates. Cytotoxicity was assessed in parallel using similar cell lines stably expressing Luc.

After two days, the reporter gene read-out of treated cells were analysed and the effect of compounds relative to untreated control was expressed as a percentage. Replication-competent and defective adenoviral type 5-based (Ad5) vectors expressing the Luc or GFP genes were similarly screened with antiviral compounds.

Results: Compounds from Aglaia species, Ramalina farinacea, Jatropha lanterossea and Nymphaea lotus displayed potent anti-HIV activity, with IC50 ranging between 2.7 and 18.2 µg/ml.

Conclusions: The recombinant viral vectors are safe and reproducibly mimick the wild-type viruses. 2) Several plant-based and two standard synthetic compounds were appropriately screened using the vector-based technique.
The Use of Permeability and Reference Compound Data for Predictions and Understanding of Human Pharmacokinetics.

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Background: Good predictions of human pharmacokinetics (PK) and drug-drug interactions (DDIs) require that permeability (Pe) is considered, as the interplay between metabolism and Pe is understood, relationships between Pe vs fraction absorbed (fa) or reabsorbed (freabs) in various important organs have been established, and that relationship compound data are available and used.

Methods: Passive in vitro Pe and in vivo PK-data were collected from the literature. These were used for establishment of Pe vs fa or freabs-relationships in the human intestines, liver, kidneys and brain, and finding suitable reference compounds for drug absorption (intestines, brain and renal), metabolism (liver and gut-wall) and excretion (renal, intestinal and biliary).

Results: Pe vs fa and freabs-relationships were established, and based on these, a Pe-based classification system (PCS) was developed. By combining the PCS and in vivo and in vitro metabolism and excretion data for reference compounds with known in vivo PK-properties, prediction of fa and freabs in various organs, hepatic clearance (CLH), gut-wall extraction ratio (EGW), excretion CL, major elimination routes, DDIs, and drug and metabolite organ/cell retention are enabled. Reference probes include atenolol (for intestinal and hepatic fa, renal freabs, EGW, gut-wall metabolism induction and inhibition potential), metoprolol (intestinal and hepatic fa, CLH, renal freabs, pharmacopoeial Pe-classification), propranolol (CLH and oral bioavailability (Fi), verapamil (EGW, CLH, and brain uptake and efflux DDI), midazolam (EGW), digoxin (intestinal, renal and biliary excretion CL, unique intestinal uptake and efflux DDI), naloxone (intestinal and hepatic absorption), rosuvastatin (intestinal excretion CL and cimetidine (renal excretion CL and end PC Pe-classification). The PCS includes 15 additional substances for Pe-categorization.

Conclusions: Pe vs fa and freabs-relationships (in the PCS) and reference compound data are believed to be very useful for improved predictions and understanding of PK and DDI-potentials in man.

Synergistic and Antagonistic Interaction between Taxanes and G1/S Arresting Agents in Combination Therapy

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Taxanes (paclitaxel and docetaxel), a novel class of naturally occurring antimitotube agents, may represent the most active chemotherapeutic agents developed in the last decade for the treatment of advanced breast cancer and many other types of solid tumors. The promising clinical profiles of taxanes have also promoted considerable interest in combining them with other therapeutic agents. However, clinical data show that such taxane-based chemotherapy or modality therapy may not always increase the therapeutic efficacy.

Through development of appropriate in vivo and in vitro model systems, we have evaluated many clinically used protocols of taxane-based combination therapy. Our results revealed that some of the taxane-containing combination therapy may result in antagonistic interactions so that the actual therapeutic activity produced by two agents is less than their expected synergistic or additive effects. Specifically, our studies show that the combination of paclitaxel or docetaxel with G1/S arresting agents such as 5-fluorouracil, doxorubicin, cisplatin, gemcitabine and gamma-radiation may produce schedule-dependent antagonistic interactions. Further, we have investigated the potential mechanism by which G1-S arresting agents interfere with therapeutic efficacy of taxanes. Data obtained from a variety of assays demonstrated that G1-S arresting agents interfere with the cytotoxic effects of taxanes on both mitotic arrest and apoptotic cell death unless taxanes are administered before G1-S arresting agents. In addition, biochemical examinations revealed that paclitaxel and docetaxel could regulate several apoptosis- and mitotic arrest-related proteins such as phosphorylation of bcl-2, -raf-1 and activation of NF-kB pathway, but these changes were inhibited when tumor cells were pretreated or simultaneously treated with G1-S arresting agents.

In conclusion, our results indicate that the interaction between taxanes and G1-S arresting agents is highly schedule dependent. Exposure of tumor cells to G1-S arresting agents before taxanes could result in pronounced antagonism. The optimal schedule for this combination might be sequential exposure to taxanes followed by G1-S arresting agents. These findings suggest that careful considerations may be necessary when combining antineoplastic agents that exert their cytotoxic action at different phases of the cell cycle.

Oxystress-induced antitumor therapeutics via targeted-inhibiting heme oxygenase-1 (HSP32) in tumor

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Background: Heme oxygenase-1 (HO-1), which is recently recognized as a heat shock protein (HSP32), play important roles in tumor growth. We have reported that HO-1 inhibitor, i.e., zinc protoporphyrin (ZnP) exhibited potent antitumor activity, however its poor water solubility hamper its application. To overcome this drawback, we prepared water soluble micelles of ZnP by use of poly(ethylene glycol) (PEG) and styrene-maleic acid copolymer (SMMAZP and S2ZP respectively) and investigated their physiochemical properties and in vitro, in vivo antitumor effect.

Methods: The molecular size and particle size of the micelles were measured by chromatography and dynamic light scattering. A rat splenic microsomal fraction was prepared for measurement of HO activity, by which the K1 was determined by using Line-weaver-Burk plots. In vitro cytotoxicity assay was carried out by MTT method; in vivo experiments were carried out by use of several tumor models.

Results: PZP and SZP showed high water-solubility (N 200mg/ml). The molecular size of the micelle is about 1444Da, and the particle size is around 60-350nm. PZP and S2ZP inhibited splenic HO activity in a competitive manner, with the K1 of 0.11mg/ml and 0.15mg/ml, respectively, which is comparable to that of native ZnP. MTX assay showed dose-dependent cytotoxicity in various cancer cells tested (average IC50 of 5µM), whereas normal cells showed relative tolerance to this treatment. In vivo antitumor experiments clearly demonstrated that PZP and S2ZP had remarkable antitumor activities, even for the highly malignant tumor-tumor Vx-2 liver carcinoma. In addition, no apparent side effects were observed in this treatment.

Conclusions: 1) Tumor-targeted inhibition of HO activity, could be achieved by using the micellar HO inhibitor based on EPR effect. Consequently, effective antitumor activity can be accomplished without any apparent toxicity in normal tissues or organs 2) PZP and S2ZP can also be applied for photodynamic therapy, which will further increase their antitumor activities.
Neuromuscular blocking drugs and magnesium interactions
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Background: Parenteral magnesium has become an increasingly popular therapeutic agent over the last fifteen years, with established uses in obstetrics, cardiology (especially management of various arrhythmias), anaesthesia and critical care. It has a number of mechanisms of action, including antagonism of the glutamate N-methyl-D-aspartate (NMDA) receptors and calcium antagonism. The latter is involved in inhibition of presynaptic release of acetylcholine (ACh). It is this mechanism that explains its action as a neuromuscular blocking agent and hence its potentiality of neuromuscular blocking (NMB) drugs. Whilst NMBs are a cornerstone of modern balanced anaesthetic practice, one of their principal side effects is persistence into the post operative period – postoperative residual curarisation (PORC). Potentiation of the effects of NMBs from any cause will make PORC more likely.

Methods: The treatment of an elective surgical patient undergoing laparoscopic cholecystectomy who developed rapid atrial fibrillation at the end of the procedure. In order to control the ventricular response rate, iv magnesium was administered.

Results: The patient had recently received NMB drugs and the co-administration of magnesium whilst NMB drugs where still present within the biophase of the neuromuscular junction lead to PORC – with the patient becoming re-paralysed. The patient required sedation and artificial ventilation of the lungs until spontaneous recovery occurred.

Conclusions: This is the first reported recuration following the administration of magnesium. Care should be exercised in the use of magnesium if NMBs have recently been administered even if the clinically normal neuromuscular function has returned. The consequences, in addition to severe distress to the patient, include paralysis and loss of airway protection.

Renal Affluent Arteriolar Vasodilator Action of Adenosine Predominantly Involves A2b Receptor Activation
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Background: Adenosine (Ado) plays an important role in regulating renal vascular tone via disparate actions of A1 and A2 receptors. While A2b receptor (A2bR) is expressed in pregolomerular vessels, there is less functional evidence regarding the role of A2bR in mediating the vasodilator action of afferent arterioles (AA).

Objective: To determine the role of A2bR in buffering the AA constriction caused by Ado by comparing the effects of A2bA and A2bA receptor blockers on AA.

Methods: We used the isolated blood-perfused juxtedullary nephron technique combined with videomicroscopy. A single AA from a rat was visualized and superfused with Ado or Ado agonist, or A2a or A2b receptor blockers (1 rat per experiment).

Results: Ado at 10µmol/L, constricted AA (-9.6±2.4%, n=5, p<0.05). In the presence of Ado, SCH, an A2a blocker, at concentrations of 1, 10, 100, 1000 and 10000 nmol/L elicited only slight decreases in AA diameter from 16.1±0.5 to 15.4±0.5, 15.1±0.4, 14.2±0.3 and 14.5±0.4µm, with maximum effect at a concentrations of 1000 nmol/L (-11.3±3.6%, n=5, p>0.05). Superfusion of Ado treated vessels with MAS, an A2b blocker, at concentrations of 1, 10, 100 and 1000 nmol/L, caused greater decreases in AA diameter from 15.7±0.5 to 14.8±0.6, 12.9±0.5 and 12.3±0.4µm (-26.0±4.7%, n=5, p<0.01). In the presence of CV101, an adenosine agonist, at concentrations of 1µmol/L, first superfusion with SCH, an A2A receptor blocker, at concentrations of 1µmol/L, AA diameter decreased slightly to 14.6±0.9µm (n=5, p>0.05). In response to CV101, an A2A agonist, at concentrations of 0.002, 0.02, 0.2 µmol/L, AA diameter increased from 17.2±0.4 to 17.1±0.4, 17.7±0.3, 18.5±0.5 and 20.1±0.3µm, with maximum effect at a concentrations of 1000 nmol/L (+11±3±3%, n=5, p<0.05). Superfusion with Ado treated vessels with MAS, an A2a blocker, at concentrations of 1, 10, 100 and 1000 nmol/L, caused greater decreases in AA diameter from 15.7±0.5 to 14.8±0.6, 12.9±0.5 and 12.3±0.4µm (-26.0±4.7%, n=5, p<0.01). Adding SCH 1µmol/L did not significantly augment the Ado mediated afferent constriction elicited by MAS 1µmol/L; however, adding MAS 1µmol/L after SCH 10µmol/L caused further vasoinvasulation with AA diameter decreasing by 16.8±2.9% from 14.8±0.4 to 12.4±0.3µm, n=5, p>0.01. In response to CV101, an A2A agonist, at concentrations of 0.002, 0.02, 0.2, 2 µmol/L, AA diameter increased from 17.2±0.4 to 17.1±0.4, 17.7±0.3, 18.5±0.5 and 20.1±0.3µm, with maximum effect at a concentrations of 1000 nmol/L (+11±3±3%, n=5, p<0.05). Superfusion with Ado treated vessels with MAS, an A2a blocker, at concentrations of 1, 10, 100 and 1000 nmol/L, caused greater decreases in AA diameter from 15.7±0.5 to 14.8±0.6, 12.9±0.5 and 12.3±0.4µm (-26.0±4.7%, n=5, p<0.01). In the presence of CV101, an adenosine agonist, at concentrations of 1µmol/L, AA diameter decreased slightly to 14.6±0.9µm (n=5, p<0.05 via MAS group). In conclusion, while both A2b and A2a receptors are functionally expressed in juxtedullary arteriolar structures, the vasodilator effect of adenosine is predominantly via activation A2b receptors.
Pivotal Evaluation of the Accuracy of a Diagnostic Biomarker: the ProBE Study Design

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Background: The Early Detection Research Network (EDRN), NCI funded and investigator driven, has the mission to evaluate biomarkers for their clinical utilities in cancer risk prediction, diagnosis, early detection, and prognosis. Abundant cancer biomarkers reported in literature yet few are used in clinics. Therefore, the emphasis of the EDRN is biomarker validation. Although schema for a phased approach to development exists and guidelines are available for study reporting, a coherent and comprehensive set of guideline for a definitive biomarker validation study design have not been delineated.

Methods: We proposed ProBE study design, Prospective specimen collection and Retrospective Blinded Evaluation, for pivotal definitive evaluation of the accuracy of a classification biomarker. A detailed formulation of all aspects of the design is provided. Four tables itemize aspects that relate to (i) the Clinical Context; (ii) Performance Criteria; (iii) the Biomarker test; and (iv) Study power and termination. Alternative designs and strategies were contrasted to illustrate the merit of ProBE design.

Results: The ideas are applied to studies of biomarkers the intended use of which is for disease diagnosis, screening, or prognosis. Two EDRN validation studies (breast cancer and prostate cancer) were used as examples to elucidate ProBE design.

Conclusion: Common biases that pervade the biomarker research literature would be eliminated if these rigorous standards were followed closely. We urge the adoption of the design as standard of practice for pivotal evaluation of the classification accuracy of biomarkers.

Smoking, Streptococci and Sputum

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Background: Smoking is estimated to cause 5 million deaths a year. The contribution of smoking to infection is less well defined than cancer and cardiovascular disease but has been shown in respiratory diseases such as influenza, tuberculosis, Legionnaire’s, *S. pneumoniae* and other infections such as *chitin media*. *H. pylori* and human papillomavirus. In 2004, Ireland became the first country to institute a ban on smoking in the workplace. This provided an exceptional opportunity to examine the effects of the ban on the epidemiology of respiratory pathogens.

Methods: The numbers of samples positive for *S. pneumoniae, H. influenzae* and *M. catarrhalis* isolated from community sputum samples were analysed for a year before and after the smoking ban. Samples from those under 18 were excluded. We obtained monthly smoking prevalence data covering the same period as the clinical data (April 2003 to March 2005) for the Munster area from the Office of Tobacco Control. Data was weighted according to the age and gender distribution of the general population. The before and after totals for each organism were compared as a proportion of the total sputum samples using the chi test. Separate logistic regression models were built for each organism under observation simultaneously adjusting for age group, gender and seasonality.

Results: 1089 and 1095 sputum samples were cultured from the community in the year before and after the smoking ban respectively. There was no significant difference between males and females for any of the organisms studied. The number of isolates of normal oral flora increased from 581 (53%) samples to 623 (57%). There was a non significant reduction in levels of all three organisms during the smoking ban. Interestingly, both *H. influenzae* and *S. pneumoniae* were reduced by the same amount (1.7%). The change in *S. pneumoniae* isolation rates approached significance (p = 0.05). On adjusting for the effects of age and gender in a logistic regression model the odds ratio (OR=0.746) for *S. pneumoniae* before versus after the ban became statistically significant (p =0.048).

Conclusions: This study showed a significant difference between *S. pneumoniae* subgroup samples before and after the smoking ban. Smoking is a risk factor for *S. pneumoniae* colonisation and infection. This is the first time that a population reduction in smoking has been shown to reduce community pneumococcal levels.

Structure-Activity Relationships of Some 1,4-Dihydropyridine (DHP) Derivatives Evaluated by Interactions with the Physical Properties of Synthetic Lipid Bilayers and Rat Liver Mitochondrial Bioenergetics

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Background: Studies of chemical–biological interactions, particularly, the influence of minor changes of substituent structure, play a major role in the understanding of drug’s structure-activity, drug’s development and therapeutic success. Aim: to correlate the length of the alkoyl chain in positions 3 and 5 of the DHP ring of four DHP derivatives (OSI-1210, OSI-1211, OSI-1212, and OSI-3852) with their actions on the physical properties of synthetic lipid bilayers and mitochondrial bioenergetics.

Methods: Biophysical studies were performed by differential scanning calorimetry (DSC) using multimellar vesicles of dimyristoyl-phosphatidylcholine (DMPC). Rat liver mitochondrial bioenergetics was evaluated by measuring respiratory activities with oxygen and tetraphenylphosphonium (TPP+) electrodes. All the experiments were performed using different DMPC and mitochondrial preparations. One-way ANOVA test, followed by the posthoc Tukey test, was used for statistical analysis.

Results: At low concentrations (≤ 30 µM), OSI-3852, like its analogue OSI-1212, reduced the phase transition temperature (Tm), the cooperative unit size and the enthalpy associated with the phase transition of DMPC bilayers. A good correlation was established between the effects of 200 µM OSI-1210, OSI-1211, OSI-1212, and OSI-3852 on the respiratory control (RCR) of rat liver mitochondria and on the enthalpy change (ΔH) for the endothermic profile of DMPC vesicles at 0.2 drug/DMPC molar ratio.

Conclusion: 1) The changes induced by these 1,4-dihydropyridine derivatives on both mitochondrial function and lipid bilayers biophysics are strongly related to the length of the alkoyl chain in positions 3 and 5 of the DHP ring. 2) This experimental strategy is a good in vitro tool to study drug structure-activity of related compounds, contributing to their synthesis amelioration.
Sero-Positivity for Hepatitis B Virus, Vaccination Coverage, and Vaccine Response in Dentists from Campo Grande, Mato Grosso do Sul, Brasil.

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Background: AIDS, caused by the virus HIV-1, is not only deadly, but becomes deadlier when the patient gets co-infected with another pathogen such as the malarial parasite Plasmodium falciparum, or Toxoplasma gondii, the causative agent of toxoplasmosis. The industry approaches to anti-retroviral drug development consist of targeting specific steps in viral entry or replication/mutation. Unfortunately, HIV-1 can quickly mutate to change such target sites, thereby becoming drug resistant. A cocktail of such individual drugs has been more effective, but does not address the problems of co-infections with unrelated pathogens. An ideal drug will be a magic bullet, that will not only simultaneously attack targets in HIV-1, P. falciparum and T. gondii, but also host factors that act as receptors of viral/parasite entry and growth.

Methods: Two bacterial proteins, azurin and Laz, members of the family called cupredoxins, showed both antiviral and antiparasitic activities. Assays of HIV-1 growth suppression, inhibition of P. falciparum parasitemia, Toxoplasma adhesion/invasion as well as protein-protein interaction studies have shown the ability of azurin and Laz to inhibit the growth of HIV-1 and malaria/toxoplasmosis-causing parasites, thus acting as a magic bullet.

Results: We demonstrate that azurin and Laz can avidly bind key envelope/surface proteins of HIV-1, P. falciparum or T. gondii, thereby interfering in their invasion of host cells and growth. Additionally, azurin/Laz strongly binds the host receptor CD4 or the dendritic cell surface protein DC-SIGN that contribute to HIV-1 transport and entry to T cells. Azurin and Laz demonstrate structural features similar to immunoglobulins, thereby implying the possibility of a common evolutionary origin of cupredoxins and immunoglobulin folds. The elucidation of broad target specificity of azurin or Laz towards viruses and parasites appears to suggest that this cupredoxin is used by the producing bacterium as a weapon targeted to other intruders in human body.

Conclusion: Our data show that azurin/Laz acts as a promising magic bullet drug candidate as conceived by Paul Ehrlich, by interfering in several essential steps in HIV-1 entry/growth and also in preventing co-infections by other human parasites.
Jasmonates Kill Cancer Cells Selectively by Dissociating Hexokinase from Mitochondrial VDAC

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Background: Cellular bio-energetic metabolism and mitochondria are recognized as potential targets for anticancer agents, due to the numerous relevant peculiarities cancer cells exhibit. Jasmonates are anticancer agents that interact directly with mitochondria. Many types of cancer cells exhibit overexpression of the key glycolytic enzyme, hexokinase, and its excessive binding to mitochondria. These characteristics are considered to play a pivotal role in cancer cell growth rate and survival. The aim of this study was to identify mitochondrial molecular targets of jasmonates.

Methods: Binding and detachment of hexokinase from mitochondria were determined by hexokinase immunochromical and activity determinations, surface plasmon resonance analysis and cellular lipid bilayer voltage dependent anion channel (VDAC)-activity analysis. Hexokinase expression was modified using hexokinase-overexpressing transfectants and its mitochondrial association.

Results: We report that jasmonate-induced detachment from mitochondria occurs in various types of cancer cells including leukemia and solid tumors. Furthermore, the susceptibility of cancer cells and mitochondria to jasmonates is dependent on the expression of hexokinase, supporting a cause and effect relationship between jasmonate-induced hexokinase detachment and cell death.

Conclusions: 1) Our findings provide an explanation for the selective effects of jasmonates on cancer cells. 2) This is the first demonstration of a cytosolic mechanism based on direct interaction between an anticancer agent and hexokinase. 3) The proposed mechanism can serve to guide development of a novel class of small anticancer compounds that kill cancer cells selectively by inhibiting the hexokinase-VDAC interaction.
Pharmacokinetics and pharmacodynamics of meloxicam in rats and humans

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Background: Meloxicam is a drug endowed of analgesic and anti-inflammatory activities. Although it is widely used in therapeutics, limited information concerning the pharmacokinetic-pharmacodynamic relationship is available. Aim of this study was to establish pharmacokinetic-pharmacodynamic modeling for antihyperalgesic and anti-inflammatory effects of meloxicam in the rat and to evaluate if levels are comparable to those reached in humans after administration of therapeutic doses.

Methods: Pharmacokinetic and pharmacodynamic evaluations were carried out in groups of rats using the paw thermal hyperalgesia and inflammation of the paw produced at much higher levels than the reached in humans at therapeutic doses. Pharmacokinetic parameters were obtained by non-compartmental approach and effects against blood concentrations were fitted to the sigmoidal Emax model.

Results: Antihyperalgesic and anti-inflammatory effects against blood concentrations of meloxicam were fitted to the sigmoidal Emax model. EC50 for antihyperalgesic and anti-inflammatory effects were 15.1 ± 2.5 µg/ml and 10.01 ± 1.76 µg/ml respectively, and Emax was in both cases about 75%. On the other hand, pharmacokinetic parameters obtained in humans were: Cmax 0.70 ± 0.03 µg/ml, t1/2 4.8 ± 0.65 h, AUC 27.7 ± 1.48 µg.h/ml and t1/2 24.5 ± 1.2 h.

Conclusions: A direct relationship between concentration and antihyperalgesic and anti-inflammatory activities of meloxicam was found, but the effect in rats is produced at much higher levels than the reached in humans at therapeutic doses.

The study of the adhesion molecules in Non Small Cell Lung Cancer (NSCLC) treated with epidermal growth factor (EGF) can generate a new drugs targets and developing new approaches for systemic treatment in lung cancer

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Background: Lung cancer is the leading cause of cancer death in developed countries. At least, six important alterations were catalogued in defined molecular events that are common to all malignant cells. Recently, several investigators attributed some six events to an important group of molecules that are important component for adherence junctions between epithelial cells (adhesion cell-cell) called caderhins and other group which is responsible for adhesion of epithelial cells to extramatrix protein (ECM) called integrins. The purpose of this study was to assess the interactions of cell adhesion molecules (CAM) in cell lines from lung cancer, where 2 of these cell lines were non-metastatic (H-358, H441) and the other two which were metastatic cells (H1299, H250).

Methods: H358 bronchioalveolar cells, H441 lung adenocarcinoma cells, H1299 and H252 lung carcinoma cells were maintained in RPMI 1640 modified medium. The cell lines were treated with epithidal growth factor (EGF) for 30 minutes. Extraction of proteins from cultured cells was performed with denaturing buffer. Protein immunodetection was done by electrophoretic transfer of SDS-PAGE, separation of proteins on nitrocellulose, incubation with antibody, and chemiluminescent second-step detection. In total of 20 proteins were performed from adhesion cell-cell and cell-extra matrix cellular (caderhins and integrons pathways).

Results: The results of cell-cell adhesion were not influenced by the treatment with EGF for 30 min. However we verified differences between the description types of cells. Otherwise, EGF could modulate the signaling pathways of the integrons and it we have show when different NSCLC cell lines are treated with EGF. We also observed antagonist functions between these proteins (PYK2 and FAK).

Conclusion: 1) The expressions patterns of adhesion cell-cell were not affect by EGF treatment, 2) The treatment can affect and may modulated the adhesion cell-ECM. 3) The antagonist effects can explain the crosstalk between EGF and integrins pathways. Some proteins searched in this study may be a key of metastatic, circulation and proliferation process in lung cancer and this may use to improve the therapeutical approaching of the NSLC.
Ixostatin, a Novel Tick Salivary Protein that Specifically Binds to the Somatomedin B Domain of Vitronectin and Prevents its Interaction with Integrin alphavbeta3 and Urokinase Receptor

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Saliva from blood-sucking arthropods is a rich source of modulators of vascular biology. In this report we describe the cloning, expression and mechanism of action of ixostatin, a novel family of 10 kDa cysteine-rich peptide from Ixodes scapularis salivary gland. Recombinant ixostatin was expressed in insect cells and was proteolytically processed into an active form. Surface plasmon resonance experiments show that ixostatin interacts with monomeric or multimeric human vitronectin with a KD ~ 0.5 nM, but does not bind to other extracellular matrix proteins. Notably, the high-affinity binding site for ixostatin was identified as the somatomedin B domain (SMBT) of vitronectin. In addition, ixostatin at nanomolar concentrations inhibits integrin alphavbeta3 and urokinase receptor-mediated cell adhesion to vitronectin, but displays negligible effects in fibroinysis in vitro. It is concluded that the most prominent biological property of ixostatin is to negatively modulate cell adhesion to the extracellular matrix. Ixostatin is the first ligand from an exogenous source that specifically targets the SMBT domain of vitronectin. It also represents a novel mechanism by which tick saliva manipulates vector-host interactions, and may therefore have potential medical applications.

Are the tropical forests the new frontier for antibiotics discovery? Novel peptidomics insights on the screening of plant antimicrobial peptides.

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Background: During human history, microbial infections directly affect World population in several areas causing economic, social, agricultural and health problems. This situation has only been controlled with the development of antibiotics. Nevertheless, the emergence of resistance has led to microorganisms’ resistance. In order to control this problem, different sources of antimicrobial peptides (AMPs) have been screened, including plants, microorganisms, amphibians, sea animals and several others. These small peptides show different and special abilities. They are able to inhibit digestive enzymes or act against bacteria or fungi.

Methods: Once those common sources have been deeply explored, in this report AMPs were screened from flowers and fruits collected from Brazilian tropical forest. In both cases were used a combo of classical strategies, which included HPLC chromatography’s and bioassays against human pathogens such as Klebsiella sp., Proteus sp. and Aspergillus fumigatus. All MICs were calculated. Furthermore novel peptidomics strategies such as liquid isoelectric focusing (pI 3-11) associated to LC-MS techniques were also utilized in order to identify antimicrobial peptides in large scale.

Results: Flowers and fruits showed AMPs from novel classes with different structures, evaluated by molecular modeling and dynamics. Among them was observed glycine-rich peptides containing per to unpunished classes. Moreover, all of them were able to cause a remarkable reduction on gram-negative and gram-positive bacteria. Additionally, it was demonstrated that it was possible to identify, by liquid IEF followed by LC-MS, at least 30 different peptides with similar properties to antimicrobial peptides, such as cationic and hydrophobic properties.

Conclusions: Current research in this area here focused, particularly aims to control pathogenic microorganisms, showing that antimicrobial peptides could be extracted and further commercialized in a near future as a common drug. Furthermore, this research also shows that tropical forests could provide new classes of antimicrobial peptides, helping to solve the infections problem.
Novel BINOL Derivatives as Photoactivatable Carriers of DNA-Targeted Potent Cytotoxic Agents

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Background: Several strategies have been developed for a selective and mild activation of DNA-modifying agents, but limited examples of photochemically activated DNA-alkylating agents have been reported. In fact, to date, porisalortes are the only well-established class of drugs known to induce DNA or RNA cross-linking upon photoactivation. Recently, our research has been focused on another class of drugs, i.e., a photoactivatable derivative of a porisalort, able to alkylate DNA.

Methods: This study included 12 healthy adult male and female volunteers to whom test and reference formulations were administered as a single-dose, 1,500 mg tablet, in a randomised, open-label, 2-week crossover fasting blood sampling design. Blood samples were collected until 12.0 hours post-dose and up to 48.00 ± 0.50 hours post-dose for mycophenolate mofetil and mycophenolic acid, respectively. Pharmacokinetic parameters AUC0-t, Cmax, and AUC0-inf, were assessed following statistical analysis with WinNonlin Pro. Population PK models were developed for mother and newborn data were performed using the nonlinear-mixed-effect modeling approach implemented in NONMEM, ADVAN5, TOLS, FIDCE INTERACTION estimation method. Final PK models were used for simulating the entire concentration-time profiles for different percentiles (P25, P50, P75) of individual PK parameter distribution.

Results: Due to sparse data, absorption rate constant was fixed to 1.66 h−1 [1]. A 2 compartment PK model was developed for mother plasma and milk data. TV (105.4 L) and CL/F (1.5 L/h) resulted in a long half-life of 49 h. Intercompartmental clearance was high (115 L/h). Interindividual variability (IV) was implemented in CL/F (28% CV). For newborn data a PK model with 'multiple input' was developed. Different input routes from mothers were combined in a boxavailability factor (18%). Plasma/milk-plasma/placenta-plasma/milk transfer rate constant, V2/F and CL/F were estimated to be 5.1 h−1, 25.8 L and 0.30 L/h, respectively, resulting in a half-life of 61 h. IV was implemented in F (93% CV) and V2/F (29% CV). Simulated concentration-time profiles revealed long-term exposures for mothers and newborns with V2/F of 10-24 and 12-22 for different individuals, respectively.

Conclusions: Population PK models for mother and newborn data were successfully developed to guide single dose NVP prevention strategies of HIV transmission from mother-to-child. 1Kappelhoff et al., Antiv. Ther., 10: 145-155 (2005).
Estrogen Receptor Beta may be a Novel Target for the Beneficial Effects of Estrogens in Females, and Androgens in Males, for Anxiety, Depression, and Cognitive Function

**Background:** Hormone-replacement therapies may have some beneficial effects for cognitive or affective processes; however, their effects and mechanisms and in the regard are not well-characterized. Studies in our laboratory have focused on the effects and mechanisms of estrogens, such as 17β-estradiol (E2), and androgens, such as the 5α-reduced metabolite of testosterone, 3α-androstenediol (3α-diol), for their anti-anxiety, anti-depressant-like, and cognitive-enhancing effects in females and male rodents. Moreover, estrogens have well-known trophic effects, which can increase risk of some cancers. As such, it is important to discern the mechanisms of steroids for their beneficial versus unwanted proliferative effects. Our laboratory has been investigating the δ isoform of the estrogen receptor (ER) as a putative target for these effects.

**Methods:** We have investigated effects on anxiety/depression and cognitive behavior utilizing the following three approaches. First, the effects of systemic or intra-brain administration of selective ER modulators (SERMs) or selective androgen receptor modulators (SARMs), which vary in their affinity for ERα or ERβ, to female and male rodents were assessed. Second, intra-brain administration of E2 and/or E2 antiserum oligonucleotides (AS-ODNs) to rats administered SERMs or SARMs was utilized. Third, the effects of SERMs or SARMs administration to mice with targeted deletions of ERα (IERKO) were assessed. Furthermore, we have determined whether some of these treatments alter tumorigenesis following exposure to a chemical carcinogen.

**Results:** Systemic or intra-brain administration of SERMs or SARMs reduces anxiety- and depression-like behavior of rats and mice. ERβ, but not ERα, AS-ODNs attenuate the beneficial effects of E2 and 3α-diol. Effects of SERMs or SARMs to reduce anxiety-like behavior and enhance cognition are not observed in IERKO mice. We have preliminary evidence about the tumorigenic role of SERMs, and have shown that E2 increases tumor burden of young ovariectomized rats.

**Conclusions:** These data support the notion that the beneficial effects of estrogens in females or androgens in males for psychological (improve affect/mood, cognition) may be via actions at ERβ.

Role of antiproliferative enzyme indoleamine (2,3)-dioxygenase in the impaired immune function in infectious diseases

**Background:** In several pathologic conditions like infections, autoimmune syndromes, cardiovascular and neurodegenerative disorders as well as malignant disease, activation and inflammation are strongly involved. Pro-inflammatory cytokines like interferon-γ (IFN-γ) play a dominant role in the clearance of infections with viruses or intracellular bacteria and parasites but also in the development of inflammation. In various cells, the expression of tryptophan-degrading enzyme indoleamine (2,3)-dioxygenase (IDO) is induced by IFN-γ as a part of its antimicrobial armature. Activation of IDO restricts protein biosynthesis by deprivation of essential amino acids and tryptophan and of tryptophan metabolism is halted. As a side effect, also development and proliferation of normal host cells like activated T-lymphocytes is diminished. Accordingly, IDO appears to represent a critical step within host-response directing whether immune activation is successful in controlling an intracellular infection or whether T-cell responsiveness is hampered, and consequently a persistent infection is developing. Increased degradation of tryptophan has been described, e.g., in patients with HIV infection, in Streptococcus pyogenes infection as well as in Lyme neuroborreliosis. Tryptophan deprivation as a result of the microbial activity of IFN-γ appears to be involved also in the pathogenesis of anemia when erythroid progenitor cells suffer from insufficient tryptophan supply. Also weight loss and cachexia are closely linked to inflammatory response when protein biosynthesis of the organism is restricted by diminished tryptophan availability. In the absence of any alternative synthetic pathways, tryptophan deprivation may serve to deplete the amino acid pool and thus may lead to reduced tryptophan availability. Both biochemical cascades seem to be involved in the development of neuropsychiatric symptoms like cognitive impairment and depression especially in patients suffering from severe and chronic infections. Thus, accelerated tryptophan degradation by IFN-γ-induced IDO can give rise to an immune activation syndrome in patients suffering from infections, which is characterized by subnormal tryptophan levels and is associated with adverse outcomes.

All abstracts are listed in alphabetical order of the presenting author.

**Abstracts**
The VesiVax® System: Vaccinology’s Magic Bullet?

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Recently, there has been significant emphasis placed on the development of vaccines that address the effectiveness, safety and manufacturing issues associated with the classical method of pathogen-based vaccines. Towards that end, the VesiVax® vaccine system was designed, which employs a flexible and easily modified gene cassette designed to rapidly engineer and produce recombinant antigen proteins that are compatible with bilayer membranes. The recombinant antigen proteins consist of a target epitope or antigen fused to an aqueous soluble hydrophobic domain that makes purification simple yet allows for stable insertion of the immunogen within the lipid membrane. Immunogenic liposomes consisting of a well-defined set of lipid constituents incorporating the recombinant antigen protein can then be produced using industry standard manufacturing processes.

Vaccines based on the VesiVax® system have been constructed against several pathogens including the influenza virus and herpes simplex type 2 virus, the causative agent of genital herpes. These vaccines have been tested in animal models and have demonstrated significant protective efficacy from microbial challenge and have elicited strong immune responses. Assays of the immunological parameters suggest that both T and B cell responses can be elicited by VesiVax® vaccines. Taken together, the inherent flexibility of the VesiVax® platform is expected to facilitate the rapid development of new vaccines which are effective at stimulating protective immune responses.

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Endovascular Stenting for Malignant Superior Vena Cava Syndrome is Essential Therapy but not Approved in Japan


Malignant superior vena cava (SVC) syndrome is difficult problems and associated with poor outcome in patients with lung cancer. Radiotherapy and/or chemotherapy could once improve the condition. Subsequently, the tumor becomes refractory and SVC syndrome appear again, constructive therapy including stent placement is abandoned since the case is viewed as terminal and treatment becomes palliative. Moreover, vascular stenting is not approved in Japan. A 66-year-old women with advanced primary lung cancer (adenocarcinoma cT1N3M1 stage IV BRA OSS) treated with 4 cycles of irinotecan/carboplatin combination chemotherapy, two times of Gamma Knife radiosurgery for brain metastasis, and radiotherapy for bone metastasis with cancer pain. Ten months later, the patient treated with oral fluoropyrimidine anticancer drug S-1 for second line, and drainage against malignant pleural effusion. For third line therapy of gefitinib, the patient maintained stable condition for a while. One year and 7 months after the onset, the patient developed severe swelling of face and both arms as SVC syndrome. We recognized that is the timing to place a self-expandable metal stent in the SVC. However, in the treatment group discussion, we attached importance to that endovascular stenting is not approved in Japan, and decided to not use the stent. The patient underwent radiation therapy (48 Gy in 20 fractions) with irinotecan (40 mg/m2/week) chemotherapy. The symptoms of SVC syndrome were resolved once, and took a turn for the worse within the chemoradiotherapy. Finally, the patient died one year and 10 months after the disease onset with miserable swollen face and both arms especially face. A post mortem examination showed complete response and almost no remaining tumor, but thrombus obstruct the SVC. Recent development of stent placement therapy for the treatment of malignant constriction has improved the quality of life, and possibly survival. In cases like our patient, chemoradiotherapy reach the limit for SVC syndrome, and Stenting is essential. The approval of endovascular stenting for SVC syndrome is warranted in the worldwide.

Chelating Agents for Treatment of Uranium-induced Toxicity in Radiation Emergency Medicine

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Background: Radiation workers treating actinides in the nuclear fuel cycle are exposed to accidental internal contamination of uranium. One of route of uranium intake is via wounds. Chelation therapy is an optimal method for reducing uranium-induced toxicity. So far, we examined on the effects of catechol-3,6-bis(methylene-imino-diacetic acid) (CBMIDA) by local treatment in simulated wounds, in which the depths contaminated with uranium in wounds are different.

Methods: Male Wistar rats, 8 weeks old, were divided into three groups (n=42/group) of intracutaneous(IC), subcutaneous(SC), and intramuscular(IM) injection. After the injection of DU (4 mg/kg, pH 1) by the different routes, rats of each group were infused with 480 mg/kg (adjusted to pH 6.8 by bicarbonate, molar rate 78 times of uranium) of CBMIDA into the DU-injected site at 10, 30, 60, 120 min and 24 hours (7 rats at each point). Rats were killed 24 hours after CBMIDA treatment. Data obtained were compared with that in the corresponding no-treated group, respectively.

Results: When CBMIDA was administered within 10 - 60 or 120 min after DU-injection, the uranium concentration of the DU-injected site decreased significantly (P<0.05) to 3-29 % (IC), 4-11% (SC), and 25-32%(IM) of that in the no-treated group. Amounts of excreted uranium increased to 4-5 times. Uranium concentrations in the kidney, as the target organ of uranium, decreased to 35-74% (IC), 22-42% (SC), and 12-39% (IM). Regarding to the kidney, as the target organ, the improvement of dysfunction by serum and urinary examinations and tissue damages by histological observation, were confirmed. Also, CBMIDA improved not only the damage by chemical action of uranium but also the burn by acid solution in the DU injected site.

Conclusions: The results indicated that CBMIDA is the useful chelating agent, (1) to increase uranium and (2) prevent the tissue damages and dysfunctions of organ, in the treatment for the wounds contaminated accidentally with uranium, if CBMIDA applies as early as possible after the intake.
Followed in the presence of 0.01, 0.1, 1, 10 and 100 promastigotes by temperatures shift from 32 to 26°C. Parasite density was determined through 14 days post infection. Infectious virus titers were determined in Madine-Darby canine kidney cells. Lung consolidation score and lung index were evaluated.

Results: Combinations of selected doses of 5, 10 and 20 mg/kg/day rimantadine and 0.1, 0.2, 0.4 and 0.8 mg/kg/day oseltamivir were combined in doses ratio 50:1. PI up to 82.7% and 91.3% and MST up to 13.2 and 13.6 days for certain combinations were evaluated, while the individual effects of the same doses were from 13.3% to 30.6% PI and 7.9 to 9.8 days MST, respectively. Determination of lung virus titers and lung parameters in combination-treated groups also proved the synergistic effect of both therapeutics. Conclusions: Oseltamivir and rimantadine at daily doses up to 50 times lower than optimal effective one for oseltamivir and 8.16 times lower – for rimantadine in 1:50 ratio demonstrated synergistic effect when administered in combination in experimental infection with influenza virus A (H3N2) in mice.

Multiple Mechanisms of Action and Pharmacological Activities of Valproate

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Background: Although it is over 40 years since the anticonvulsant properties of valproate were discovered serendipitously, this drug remains one of the most widely prescribed anti-epileptics. Interestingly, several other indications have since emerged for valproate. In addition to these therapeutic effects, valproate is a known teratogen inducing neural tube defects in exposed offspring – an effect that is largely reflective of its anticonvulsant properties, treatment of mania and, most recently, chemotherapy of acute myeloid leukemias. In addition to these therapeutic effects, valproate is a known teratogen inducing neural tube defects in exposed offspring – an effect that is largely reflective of its anticonvulsant properties, treatment of mania and, most recently, chemotherapy of acute myeloid leukemias.

Methods: The influence of valproate on cell proliferation, differentiation and cell cycle signaling was investigated in glioma (C6) and neuronal cell lines. These signaling pathways, along with other proposed mechanisms of action that may mediate the antiproliferative activity of valproate are reviewed and discussed.

Results: Valproate arrested cell cycle progression, induced ectopic expression of cyclin D3 and inhibited the accumulation of cyclin regulation by K-ras and p53-dependent processes. While the precise mechanisms of action that underlie these distinct pharmacological activities are unclear, several different signaling pathways are influenced by valproate and related drugs.

Conclusions: The influence of valproate on cell proliferation, differentiation and cell cycle signaling was investigated in glioma (C6) and neuronal cell lines. Cell cycle synchronization was achieved by mitotic selection and western blotting techniques were employed to investigate the expression of cyclins and related proteins. The effect of valproate on cell cycle signaling was investigated in forskolin-treated cells. These signaling pathways, along with other proposed mechanisms of action that may mediate the antiproliferative activity of valproate are reviewed and discussed.

Authors’ disclosure statement: The author’s investigations into the mechanism of action of valproate have been financially supported in part by the Spanish biopharmaceutical company Pfizer.

Perspective Chemotherapeutic Combination to Combat Flu

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Background: Previous studies of ours demonstrated a marked synergistic combination effect of rimantadine and oseltamivir in 100:1 compounds doses ratios in experimental infection with influenza A (H3N2) in mice when the treatment course onset was on the day of virus inoculation. Considering these data we studied combination effect of both compounds in 50:1 and 25:1 ratios in order to determine the dose ratios scope preserving a high efficacy. The antiviral effect of the treatment course with the combination started 24-hours after virus inoculation was tested.

Methods: Male white mice 16-18 g were inoculated intranasally with 0.05 ml/mouse of influenza A/Avicri/2/68 (H3N2) virus. Rimantadine hydrochloride and oseltamivir phosphate were administered per os in five-day-treatment course beginning 4-hours before or 24 hours post-virus inoculation with 20–30 MLUD Protection index (PI) and mean survival time (MST) were determined through 14 days post infection. Infectious virus titers were determined in Madine-Darby canine kidney cells. Lung consolidation score and lung index were evaluated.

Results: Combinations of selected doses of 5, 10 and 20 mg/kg/day rimantadine and 0.1, 0.2, 0.4 and 0.8 mg/kg/day oseltamivir were combined in doses ratio 50:1. PI up to 82.7% and 91.3% and MST up to 13.2 and 13.6 days for certain combinations were evaluated, while the individual effects of the same doses were from 13.3% to 30.6% PI and 7.9 to 9.8 days MST, respectively. Determination of lung virus titers and lung parameters in combination-treated groups also proved the synergistic effect of both therapeutic agents as well as prevention of influenza-like symptoms.

Conclusions: Oseltamivir and rimantadine at daily doses up to 50 times lower – than optimal effective one for oseltamivir and 8.16 times lower – for rimantadine in 1:50 ratio demonstrated synergistic effect when administered in combination in experimental infection with influenza virus A (H3N2) in mice.

Abstracts Page A-100

All abstracts are listed in alphabetical order of the presenting author.
The photophysical properties of the promazine family depend more on solvent and the 2-substituents than on the diaxylaminopyrrol chain. The largest effect was found for the triplet state of the 2-halogenated derivatives in the photodynamic range. The rate of this quenching correlates with the occurrence of heavy atom effects.

Methods: The photophysical properties were measured in several solvents. In this work, we present absorption, steady-state, and time-resolved emission, laser flash photolysis, and quantum theoretical results for the ground state, the first excited singlet and triplet states, and the cation radical of several TCA series.

Results: The photophysical properties of the promazine family depend more on the solvent and the 2-substituents than on the diaxylaminopyrrol chain. The largest effect was found for the triplet state of the 2-halogenated derivatives in the photodynamic range. The rate of this quenching correlates with the occurrence of heavy atom effects.

Conclusions: 1) The effectiveness of the TCA* quenching in PBS correlates very well with their photooxidizing toxicity (i.e., the more effective the quenching, the smaller the triplet lifetime and the more photooxidic the drug). 2) Besides the TCA*, the involvement of some membrane components is required to explain the large differences in photooxidic toxicity of similar TCAs.

Antimicrobial and liposomal carriers as novel strategies for chemotherapeutic therapy based on cisplatin in cervical cancer.

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Background: Cisplatin (CP) is a widely used antineoplastic drug that has potent cytotoxic effects upon a variety of tumor types including cervical carcinoma. However, its administration is associated with nephrotoxic and neurotoxic events. On the other hand, steroid hormones are related to the development of drug resistance in cervical cancer. Upon this situation, in this work we have investigated the ability of a pure anti-estrogen ICI 182,780 and an anti-progestin Mifepristone (MF) to modulate the cytotoxic effect of CP and gamma irradiation in cervical cancer cell lines (HeLa and CaSki) and in a model of cervical cancer in athymic mice.

Methods: The effect of CP alone and CP combined with either ICI, MF and/or 7% ethanol was evaluated with dosimetric procedures based on Gafchromic film. The administration of a pure anti-estrogen ICI 182,780 (Fulvestran) and an anti-progestin Mifepristone (MF) was evaluated with dosimetric procedures based on Gafchromic film. The administration of ICI or MF alone produced no changes in cell survival and an additive effect of CP and MF compared to the results obtained with either compound alone. In contrast, the combination of antihormonal drugs produced an enhanced cytotoxic effect. The combination of antihormonal drugs with CP produced a supra-additive antiproliferative effect in cervical cancer cells and significant delayed of the tumor growth without apparent toxic effect for the animals (p<0.05, n=6). The administration of CP and MF alone or in combination did not produce toxicity in the normal tissues evaluated, at least partially, by inhibition of ER, PR and VEGF gene expression, and by arresting the cell cycle at G2/M phase.

Conclusions: The results suggest that the combination of antihormonal drugs can improve the efficacy of CP and RT in cancer cells and tumor xenografts of cervical carcinoma. Based on these results we have planned the use of liposomes as drug carriers of the CP and MF, which potentially could be used in chemoradiotherapy treatments decreasing the secondary effects of these drugs.
A Climate for Change? A Statistical Analysis of General Practitioners’

Relationship with Chiropractic Care.

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Introduction: Chiropractic is the complementary medicine dealing with correcting misaligned joints through mechanical manipulation. This study aims to identify the knowledge, attitudes and referral patterns of general practitioners (GPs) in Fife, Scotland regarding chiropractic care.

Materials and Methods: A postal questionnaire was sent to 100 GPs in Fife. Reply-paid envelopes were included, and a reminder questionnaire sent out one week later. GPs from ten randomly selected practices were asked to participate in a follow-up interview.

Results: Despite evidence demonstrating the usefulness of chiropractic, over 80% of GPs rated their knowledge of chiropractic as less than or equal to five out of ten (with 10 representing ‘very knowledgeable’). The average score was three. GPs’ attitudes towards chiropractic were fairly positive, though these attitudes were not reflected in GP perception of the helpfulness of chiropractic, or in their referral practices. In general, 3% of GPs found chiropractic very helpful, 14% rated it as helpful, 12% as neutral and 72% as unhelpful. GPs referred most readily for lower back pain, neck pain and sciatica. On average, 3% of GPs refer patients to chiropractors at first consultation and 9% after failure of traditional treatments. 10% only at the patient’s request, and 76% would never refer. Thirty-six percent of GPs never refer patients to a chiropractor for any condition.

Conclusion: The results indicated an under-utilization of chiropractic treatment. GPs’ attitudes towards chiropractic were positive; however, attitudes did not show a strong correlation with referral practices or perceptions of helpfulness. GPs’ perceptions of the conditions treated by chiropractic medicine do not match the evidence in this field. In general, GPs under-valued the helpfulness of chiropractic. However, for some conditions GPs perceived chiropractic to be more helpful than evidence-based medicine research supported. How helpful GPs perceived chiropractic to be was strongly correlated with the stage at which they were willing to refer patients to chiropractors. GPs seem very unwilling to refer patients to chiropractors; most will never refer or only refer at the patient’s request.

Recombinant virus-like particles and their application for vaccine development and diagnostics

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Background: Supermolecular structures, named VLPs (virus-like particles), built symmetrically from hundreds of proteins of one or more types, represent molecules useful for the development of diagnostic, prophylactic and therapeutic tools for fighting human diseases. The aim of this study was to adapt the yeast expression system for poliovirus and paramyxovirus nucleocapsid protein (NP) expression and use it for VLP production. Methods: VLPs for diagnostics, monoclonal antibody generation, immunological investigations or virus basic research applications, as structural and assembly studies, receptor identification and structural elucidation, were used for enzyme immunoassay studies, mice immunizations and monoclonal antibody generation using hybridoma technology.

Methods: A galactoseinducible S. cerevisiae yeast expression system was used. For induction of empty VLPs was confirmed by cesium chloride ultracentrifugation, agarose gel electrophoresis and electron microscopy. Recombinant VLPs were used for enzyme immunoassay studies, mice immunizations and monoclonal antibody generation using hybridoma technology.

Results: The high efficiency of the S. cerevisiae-based expression system was confirmed by the production of VLPs based on the VP1 of different human (JCPyV, BKPyV), primate (SV-40, B19V), mouse, hamster and avian polyomaviruses. The expression level of most polyomavirus VP1 proteins and mumps virus NP proteins encoded in E. coli systems were used for structural and assembly studies and as molecular carriers of selected epitopes for production of chimeric VLP and the monoclonal antibody of desired specificity.

Conclusions: 1) We developed a universal expression system in yeast, for poliovirus and paramyxovirus nucleocapsid protein synthesis and VLPs production. 2) The yeast generated recombinant virus VLP retain biological activity of native virus proteins and were successfully used for detection and generation of specific antibodies.3) Polyomavirus VP1-VLPs were used for diagnostics, virus structure and entry studies and chimeric virus-like particles production. Development of Serpin drugs for the treatment of HIV/HCV co-infections

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Background: Novel antivirals against HIV and HCV targeting host-cell proteins are needed to prevent the generation of multi-resistant viruses. The Serpine protein inhibitors (Serpins) such as Sereoticya Leuococyte Protease Inhibitor (SLIP), anti-thrombin III (ATIII) and Anthrombin III (ATIII) all display potent antiviral activity against HIV in vitro. Their in vivo potential can be demonstrated by: a) the near-absense of HIV oral transmission most likely due to the anti-viral activity of SLIP, the predominant HIV-inhibitor in saliva; b) the correlation between disease progression and certain anti-trypsin mutations; c) the observation that CDF T cells of HIV-long term non-progressors produce a high amount of ATIII with high anti-viral activity. STUDY AIM: 1) Demonstrate anti-viral inhibition of ATIII for HIV and HCV; 2) Elucidate novel host-cell target-attains for ATIII is the first recombinant protein produced in goats and approved for human use. Due to its improved availability, 60 h half-life and low toxicity ATIII has strong potential as a novel protein-based anti-viral against HIV in a multi-resistant phenotype.

Methods: HIV inhibition was measured in cell lines and human Peripheral Blood Mononuclear Cells (PBMC). HCV inhibition was measured using a replicon system with a full-length HCV genome. Activation or inhibition of pathways and host-cell targets was measured by microarray with 84 key genes testing for 18 different pathways.

Results: ATIII blocked HIV viral replication in nM and HCV in μM concentrations in a dose dependent manner. Using 2.4, 12 and 24 U/ml ATIII we saw 8 genes in HIV infected PBMC upregulated (Prostaglandin-endoperoxide (PTGS2), IL-8, IL-1α, IL-1β, IκBα, TNFα, IL-8 and IL-1α were both 60-fold upregulated. In the HCV replicon system these seven genes were more than 10-fold downregulated. Cancer genes Jun and Myc where up to 1000-fold and 60-fold downregulated, respectively. transfection factor C/EBP was downregulated more than 600-fold.

Conclusions: ATIII blocks viral replication through a mechanism of action that targets multiple host-cell proteins which might decrease the ability of the viruses to develop resistance to this modality.
Adoptive immunotherapy with Streptamer-selected HCMV specific T-cells after allogeneic stem cell transplantation

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Abstracts

Development of Nonviral Gene Vectors for Gene Delivery to the Lungs and Blood Cells

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Background: Nonviral gene therapy could offer the opportunity to cure various inherited and acquired pulmonary and hematopoietic diseases. The aim of gene therapy is to (1) design effective nonviral gene vectors, (2) optimize efficiency and specificity of transfer of genetic material into target cells, (3) minimize adverse toxicity and immune responses, (4) maximize the therapeutic potential.

Methods: Nonviral vectors, both episomal-explicating (SIMAR) and integration (\(\Phi\)C31 integrase) based, were designed. Well-defined gene transfer agents possessing biodurability, targeting functionalities and reduced toxicity were synthesized and applied via different routes into mice. Aerosol-based targeting of lung regions by combining gene vectors with magnetic gradient fields was investigated in vivo.

Results: Stable gene expression was achieved using different nonviral vectors together with plasmid DNA containing UbiC or Ubiquitin B promoters and S1MAR elements in hematopoietic cells in vitro. Stable expression in the lungs of mice was obtained with the co-delivery of \(\Phi\)C31 integrase expression plasmid.

When delivered as a fusion protein, recombinant \(\Phi\)C31 integrase-TAT, mediated site-specific recombination in mammalian cells in vitro. Using lactoferrin, insulin and chimerist as ligands coupled to PEI, selectivity towards specific cell types was achieved. Intracellular trafficking of the plasmid into the nuclear matrix, resulted in higher and stable long-term expression in lungs in vitro. High toxicity and non-robust biodurability of PEI limit its in vivo application. Therefore, well-defined polymethacrylate based copolymers were characterized as gene transfer agents with low cytotoxicity, high colloidal stability and comparable transfection efficiency in vitro. Efficient methods for delivery of gene vectors to localized regions of the lung have been successfully established in mice by application of an extended gradient magnetic field during inhalation of aerosols containing superparamagnetic iron oxide nanoparticles.

Conclusions: The standard requirements for clinical use of nonviral vectors have not been met yet in terms of efficiency and specificity. Future research will focus on improving the efficiency, specificity and safety of the gene delivery systems.

Phototherapy as a new method for the treatment of cutaneous leishmaniasis

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Background: Leishmaniasis is an important disease caused by Leishmania spp. This disease occurs within a few weeks after a small papule on the exposed site and finally ulcerates. The drugs of choice, pentavalent antimony and amphotericin B, are characterized moderately toxic and there are risks of recurrence and unsatisfactory side effects. Leishmania was found deficient in at least five enzymes in the bame biosynthesis pathway. The first enzymes were phenylalanine deaminase (ADA) and delta-aminolevulinic acid (ALA). During the irradiation with red light the porphyrin-synthesizing enzymes were activated and finally the porphyrin-enriched tissue was killed.

Methods: In this study, we used photodynamic therapy (PDT) for the treatment of cutaneous leishmaniasis caused by leishmania major.

In this study, the Leishmania lesions of five patients was applied locally with ALA 10%, then after 4 hours per treatment session was delivered, using red light (570–670 nm), 100 µmol/cm² at a light intensity of 150 mW/cm² (approximately 21 min). Treatments were repeated weekly for 4 times.

Results: In direct staining smears were showed no amastigotes after one or two sessions. The follow up continued for four months. The results showed that photodynamic therapy to modeling of lymphocytes and macrophages.

Conclusion: Treatment of cutaneous leishmaniasis is targeted toward the eradication of amastigotes and the reduction of the size of the lesions to promote healing and achieve maximum efficacy with minimal scarring and toxicity. Photodynamic therapy in contrast to all systemic treatment modalities has no risk of toxicity but only mild local inflammatory reaction with an excellent cosmetic outcome. So PDT might offer a new promising treatment modality for the disseminated lesions of cutaneous leishmaniasis.
Designing Material of Particular Equilibrium and Transport Properties by Ab Initio Molecular Dynamics

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Background: Prediction and study of equilibrium and transport properties of ionic liquids as green solvent are currently of high interest. These liquids are used in organic synthesis without harmful environmental effect of ordinary volatile solvent. Application of atomistic simulation methods to design 1-alkyl-3-methyl imidazolium based ionic liquids of suitable equilibrium and transport properties as well as understanding the aggregation mechanism within the liquid ion liquid assembly forms the aims of the present study.

Methods: ab initio Car-Parrinello molecular dynamic (CPMD) simulation was used to simulate the transport properties including viscosity and diffusion constant of 1-alkyl-3-methyl imidazolium based ionic liquids. Simple anion like chloride (Cl-) and iodide (I-) and complex ones like BF4- and PF6- was studied. All the simulations were made at 300K. In the same way the aggregation of the ionic liquids with long chain alkyl group was studied. A thorough understanding of the dynamic and the structure were followed by studying the static properties by using Gaussian program.

Results: Structural and the dynamic properties were studied by the results of simulation based on statistical mechanics of the liquid state. The studies show a slow decrease in the viscosity with increasing temperature and with the number of carbon compounds than the iodide one. The I- compound form larger aggregates than the Cl- one. Contrary to the ionic liquids with Cl- and I- anions, the BF4- and PF6- compounds show hydrophobic properties, and therefore they show higher dynamics, smaller viscosity, and those properties characteristics of tightly bonded ion pair salts.

Conclusions: The slow dynamics and high viscosity of the imidazolium based ionic liquids containing simple anions are due to the strong electrostatic cation-anion interaction. The more complex anions make a more complex interaction leading to the hydrophobic property. The details knowledge of these properties enables to design system of interest with desire equilibrium and transport characters.

Evidence for Anti-Cancer activity for the Antidepressant Sertraline, In-Vitro and In-Vivo Effect in Nucle Mouse Xenografted with HT22 cells.

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1Lab Biological Psychiatry, Tel-Aviv University, Felsenthal Institute, Campus Rabin, Peto-Tixava, Israel, and 3Perigo Israel Pharmaceuticals Ltd. Bnei Brak, Israel

Background: Recent reports provide evidence for a pro-apoptotic activity of some SSRIs in different tumours. A possible explanation of this activity is the effect of the SSRIs on apoptosis markers. 2. Determination of the molecular mechanism of the drugs. 3. Evaluation of the in vivo effect of the SSRI's in a mouse model xenografted with human colorectal carcinoma cells HT22.

Methods: Human colorectal carcinoma HT22, and LS1034, a multi drug resistant (MDR) cell lines (ATCC) were studied. Cell viability (neutral red) and cell proliferation (thymidine incorporation) were determined. Apoptosis was studied using flow cytometry of propidium iodide stained cells and caspase 3 determination by an enzymatic fluorometric assay. Protein expression was determined by western blot analysis. Tumor growth was determined in CD1 nude mice xenografted with the drugs.

Results: The SSRIs paroxetine and sertraline induced a dose-dependent inhibition of cell viability and proliferation in both cell-lines (IC50 8-15mM). When compared to some cytotoxic agents e.g doxorubicin, vincristine and 5FU, the SSRi's activity demonstrated a similar (HT22) or stronger effect (LS1034). Both agents stimulated DNA fragmentation and increased caspase-3 activation, suggesting a proapoptotic mechanism. Western blot analysis revealed an increase 24hr later in c-Jun and p-ERK and decreased Bcl2 expression. For in vivo experiments, we used CD1 nude mice xenografted subcutaneously with HT22 cells. Sertraline (3 times/week 15mg/kg s.c or i.p.), but not paroxetine, induced a significant inhibition of tumor growth and survival.

Conclusions: Collectively, our results suggest that the widely used and safe antidepressant sertraline possesses potential anti-tumor activity, which circumvents the MDR mechanism and thus could be valuable in the arsenal of colon carcinoma therapy. Since SSRI therapy is frequently indicated in cancer patients as an antidepressant, this possibility seems attractive.

Treatment of Intravenous Drug Users with Chronic Hepatitis C: Treatment Response, Compliance and Side Effects

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Background: Although IVDUs comprise the majority of patients with chronic hepatitis C, most of them are excluded from treatment because of concerns about adherence to treatment and side effects.

Methods: In this study we retrospectively evaluated safety, compliance to treatment and efficacy of treatment in IVIDUs with HCV infection in 163 former IVIDUs with chronic hepatitis C, who were not in methadone substitution and were attending our clinics during 1997-2004. All subjects were HCVRNA (+), had ALT levels >X1,5 UNL and were treated for their HCV infection. Treatment consisted of IFN- α monotherapy (39,8%), IFN-α/ribavirin combination therapy (30,1%) and pegylated IFN-α/ribavirin combination therapy (67,163 patients (53,3%) discontinued treatment early due to drug adverse relapse (62%), side effects (32,1%, 10% psychiatric) and 5,7% for other reasons. 80% of those who discontinued treatment had pre-treatment drug abstinence and 20% of them relapsed to drug use. We suggest that the optimal duration of pretreatment drug abstinence should be 9 months.

Results: ETR and SVR were significantly higher in both combination therapies compared to IFN-α monotherapy. The most prevalent HCV genotype was 3 (65%) and mild histological lesions were detected in the majority of subjects. In conclusion our findings show that treatment for chronic hepatitis C was reasonably safe and sufficiently effective in our group of non methadone-substituted IVDUs, despite the fact that more than half of them discontinued treatment early and many relapsed to drug abuse. We suggest that the optimal duration of pretreatment abstinence from drug abuse should be 9 months.
Serotonin Transporter: Mechanisms of Inhibition by Enteropathogenic E. coli (EPEC)

GILL R., ESMAILI A., NAZIR S., BORTHAKUR A., TURNER J., ALREFAI W., HECHT G., DUDEJA P.

Background: Serotonin transporter (SERT) activity plays a critical role in regulating serotonin availability by its uptake through a Na+ and Cl− coupled mechanism. Elevated levels of serotonin are associated with several diarrheal conditions including inflammatory bowel diseases and enteric infections. However, whether alteration in SERT activity contributes to the pathophysiology of diarrhea induced by food-borne pathogens such as enteropathogenic E. coli (EPEC) is not known. EPEC is non-toxigenic but houses a pathogenicity island encoding a type III secretion system (T3SS) that translocates bacterial proteins directly into host cells. We hypothesized that EPEC decreases SERT activity to contribute to the associated rapid diarrhea. The present studies were aimed at examining the effects of EPEC infection on SERT activity and expression and delineating the underlying mechanisms.

Methods: Caco-2 cells were used as an in vitro model of human intestinal epithelia and were infected with EPEC strain E2348/69 or commensal E. coli. SERT activity was measured as fluvoxamine-sensitive [3H]-5-HT uptake. Infection of Caco-2 cells with EPEC for 12.05:30 min decreased luminal SERT activity (~50-60% inhibition at 30 min; *P<0.005); however, infection with commensal E. coli had no impact. Kinetic analysis revealed that EPEC infection inhibited SERT activity via a decrease in Vmax (~ 3 fold). In parallel, parallel, EPEC infection caused internalization of SERT from the plasma membrane to endocytic vesicles as assessed by live cell imaging of SERT-GFP construct transfected Caco-2 cells. Mutation of esoc5, which encodes the ATPase for T3SS, ablated the effect of EPEC on luminal SERT activity indicating that effects of EPEC were T3SS dependent. Inhibitory effect of EPEC on SERT activity was abolished in the presence of tyrosine phosphatase activity indicating that effects of EPEC were T3SS dependent. Inhibitory effects of EPEC in infection on SERT activity and expression and delineating the underlying mechanisms.

Conclusions: Infection of intestinal epithelial cells with EPEC decreases SERT via a T3SS-dependent mechanism and involvement of tyrosine phosphatases. These data further highlight the interactions of a common enteric pathogen with the expression and function of SERT and provide mechanistic insights into development of a potential new pharmacotherapy to modulate the serotonergic system in treatment of diarrheal diseases.

Erythropoietic Stimulating Proteins - What is the Optimal, Safe Hemoglobin Target?

GILMARTIN C

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Background: Erythropoietic stimulating proteins (ESPs) have been used in the treatment of anemia of chronic kidney disease (CKD) and chemotherapy-induced anemia (CIA) since 1989 and the early 1990s, respectively, to a target hemoglobin (Hgb) between 11 to 12 g/dL. Recent clinical trials have examined Hgb targets > 12 g/dL that have demonstrated increased mortality and thrombotic events in both anemia of CKD and CIA. ESPs have also been utilized for the off-label indication of anemia of cancer.

Methods: A review of the clinical trials prompting the addition of the boxed warning to the ESPs product labeling was performed. An evaluation of the trials including target Hgb, primary endpoints and outcomes in both CKD, CIA and anemia of cancer was assimilated.

Results: The following table summarizes the trials in CKD anemia.

<table>
<thead>
<tr>
<th>Reference (Study)</th>
<th>High Target</th>
<th>Primary End Point</th>
<th>Outcome</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardeña et al. 2003 (1)</td>
<td>Hgb = 13.5</td>
<td>Complete death or myocardial infarction (MI) or congestive heart failure without death</td>
<td>Complete death or myocardial infarction (MI) or congestive heart failure without death greater than or equal to group treated by ESPs</td>
<td>10.0</td>
</tr>
<tr>
<td>Cardeña et al. 2004 (CREATE)</td>
<td>Hgb = 13.5</td>
<td>First cardiovascular (CV) event</td>
<td>No benefit in ESPs to first CV event</td>
<td>1.20</td>
</tr>
<tr>
<td>Harada et al. (1998)</td>
<td>Hgb = 12.0</td>
<td>42% death or death due to cancer (encephalopathy)</td>
<td>42% death or death due to cancer (encephalopathy)</td>
<td>4.48</td>
</tr>
</tbody>
</table>

The table below summarizes the trials for CIA and anemia of cancer.

<table>
<thead>
<tr>
<th>Reference (Study)</th>
<th>Target</th>
<th>High Target</th>
<th>Primary End Point</th>
<th>Outcome</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoffstetter et al. 2004 (IN HCT)</td>
<td>Hgb = 12.0</td>
<td>Complete death or death due to cancer (encephalopathy)</td>
<td>Complete death or death due to cancer (encephalopathy)</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>Cardeña et al. (2003)</td>
<td>Hgb = 13.0</td>
<td>Complete death or death due to cancer (encephalopathy)</td>
<td>Complete death or death due to cancer (encephalopathy)</td>
<td>1.20</td>
<td></td>
</tr>
</tbody>
</table>

High-dose cyclophosphamide is active in immune-mediated illnesses.

GLADSTONE D

Stony Brook University, New York, USA

Background: High-dose cyclophosphamide is active in immune-mediated illnesses.

Objective: To describe the effects of high-dose cyclophosphamide on severe refractory multiple sclerosis.

Design, Setting, and Patients: Patients with multiple sclerosis with an Expanded Disability Status Scale (EDSS) score of 3.5 or higher after 2 or more Food and Drug Administration-approved disease-modifying therapy regimens were evaluated.

Intervention: Patients received 200 mg/kg of cyclophosphamide over 4 days.

Main Outcome Measures: Patients had brain magnetic resonance imaging ad neuro-ophthalmologic evaluations every 6 months and quarterly EDSS and quality-of-life evaluations for 2 years.

Results: Twelve patients were evaluated for clinical response (median follow-up, 15 months; follow-up range, 24 months). During follow-up, no patients increased their baseline EDSS scores by more than 1.0. Five patients decreased their EDSS scores by 1.0 or more (EDSS score decrease range, 1.0-5.0). Two of 11 patients had a single enhancing lesion at baseline; these lesions resolved after high-dose cyclophosphamide treatment. At 12 months, 1 patient showed a new enhancing lesion without a corresponding high-intensity T2-weighted or fluid-attenuated inversion recovery signal. Patients reported improvement in all of the quality-of-life parameters. Neurologic improvement involved changes in gait, bladder control, and visual function. Treatment response was seen regardless of the baseline presence or absence of contrast lesion activity. Patient quality-of-life improvement occurred independently of EDSS score changes. In this small group of patients with treatment-resistant severe refractory multiple sclerosis, high-dose cyclophosphamide was associated with minimal morbidity and improved clinical outcomes.

Conclusions: High-dose cyclophosphamide treatment in patients with severe refractory multiple sclerosis can result in disease stabilization, improved functionality, and improved quality of life. Further studies are necessary to determine the most appropriate patients for this treatment.
Intravascular fluid replacement in the critically ill: Is it the substance or the timing that makes the difference?

GOEFPERT MS, KUBITZ JC, GOETZ AE, REUTER DA

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Background: The ideal infusion solution used for intravascular fluid replacement in critically ill patients is still not determined in order to improve patients outcome. Further, inconsistent opinions exist whether restrictive or liberal fluid management is beneficial in critical ill patients. In this study we aimed to analyze patient characteristics, and how to guide fluid therapy in critically ill patients has not been elucidated yet satisfactorily. We therefore performed a prospective study in cardiac surgery patients where the timing and the amount of fluid replacement was guided by an early goal directed hemodynamic algorithm using volumetric parameters of cardiac preload for optimization of timing and quantity of intravascular fluid replacement.

Methods: 40 patients undergoing cardiac bypass surgery were included prospectively (study group, SG) and compared with a control group (CG). In the SG, from induction of anesthesia until 48 hours after surgery, hemodynamic management was continuously guided by an algorithm based on the measurement of global end systolic volume (GEVS) and cardiac index (CI). Hemodynamic goals were a GEVS > 640 ml/m², CI > 2.5 l/m², and mean arterial pressure (MAP) > 70 mmHg. The CG was treated at the discretion of the attending physician based on central venous pressure (CVP), MAP and subjective clinical evaluation.

Results: Total duration of catecholamine and vasopressor dependency was significantly shorter in the SG (187 ± 70 min vs. 1458 ± 197 min, p < 0.001). Less vasopressors (0.73 ± 0.32 mg vs. 6.67 ± 1.21 mg, p < 0.001) as well as catecholamines (0.01 mg vs. 0.01 mg, p = 0.83 vs. 0.027 mg, p < 0.001) were administered in the SG. In tiostatid infusions no differences were detected at any time. In the SG significantly more clopidogrel (HES 130:0:4, gelatin solution 3.5%) were used (151.5 ± 60 ml of clopidogrel vs. 132.7 ± 59 ml during surgery and 5403 ± 222 ml vs. 4187 ± 167 ml during ICU therapy). Comparing all 6 cases of fluid replacement (6509 ± 240 ml SG vs. 6403 ± 184 ml CG, p = 0.05) there was no clinical relevant difference. SG patients reached ICU-discharge criteria significantly earlier (25 ± 13 vs. 33 ± 17 h).

Conclusions: Not only the substance used for fluid replacement but continuous preload optimization and the optimal timing for fluid application are necessary to improve critically ill patients outcome.

Antibody responses in cancer vaccines and immunotherapies: from cancer/testis antigens to new targets discovered by protein arrays

GNAJIC S, ODUNSI K, ALTORKI NK, RITTER G, BUECHLER MW, JAEGER D, OLD LJ

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Background: Analysis of antibody responses to self-antigens has driven the development of the field of tumor immunology. While cancer/testis antigens have been put to the test in clinical trials, more targets are needed. Protein microarrays technologies offer an unprecedented platform to assay the serological response of cancer patients to tumor antigens in a comprehensive fashion.

Methods: Sera from patients with non-small cell lung cancer (NSCLC), epithelial ovarian cancer (EOC), and pancreatic cancer, as well as healthy donor sera from the New York Blood Bank, were collected and tested by Enzyme-Linked Immunosorbent Assay (ELISA) against known recombinant tumor antigens, as well as by antibody profiling using commercially available protein microarrays containing >20,000 antigens.

Results: We first validated our approach by using sera with known immunoreactivity in ELISA to antigens present on microarrays. We found 197 antigens reacting frequently and strongly with EOC patient sera compared to healthy donor sera. The same study with pancreatic cancer patient sera returned 28 antigens with preferred immunoreactivity in cancer, 21% of which overlapped with antigens immunogenic in EOC.

Conclusions: With a stringent strategy for data analysis and normalization our approach of using antibodies of patients to tumor antigens present on protein microarrays, we describe new antigens immunogenic in cancer patients and propose that this approach is suitable for defining potential antigenic targets for cancer vaccine development, serum antibody signatures with clinical value, characterization of predictive serum markers for experimental therapeutics, and eventually for the serological definition of the cancer proteome (seromics).

Changing the dosing frequency of cetazidime transforms this “Daisy cutter” into an antibiotic with limited collateral damage while retaining its efficacy

GOEFPERT MS, KUBITZ JC, GOETZ AE, REUTER DA

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Background: The occurrence of vancomycin resistant Enterococcus (VRE) has been increasing from 2.7% to 13.5% in Germany (2002-2004). This is due to too high, too low and inadequate dosing of antibiotics.[1] For VRE colonized patients linezolid offers an alternative treatment. The prediction of the antibacterial effect by a pharmacokinetic/pharmacodynamic (PK/PD) analysis could guide the antibiotic therapy, shorten the duration of disease and reduce the extent of bacterial resistance.

Methods: The killing behaviour of VRE (ATCC 700221) over time under various linezolid concentrations was investigated in a static in vitro model. An inoculum of 10^8 cfU/mL VRE in Mueller-Hinton (MH) broth was spiked with linezolid concentrations of 0.5, 1, 2, 4, 8, 16, 32 µg/mL, incubated at 37 °C and continuously shaken (62 min⁻¹). The time-killing process was monitored via viable cell counting over 24 h. Bacteria samples were taken at 0, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h and plated on MH agar plates with 5% sheep blood. After 24 h of incubation all plates were counted by a digital automatic colony counter ( ColonyQuant, Schuett Biotec, Goettingen, Germany). Geometric means and confidence intervals of the respective bacterial concentrations were numerically calculated via bootstrapping in Excel (Microsoft).

Results: The time-kil curves of VRE under various linezolid concentrations were investigated. Linezolid concentrations of 4 and 8 µg/mL were identified as bacteriostatic and 17 and 36 µg/mL as bactericidal. For concentrations lower than 4 µg/mL, bacterial regrowth after 24 h was observed. However, 17 and 36 µg/mL achieved very similar rates in bacterial killing.

Conclusions: Longer-term in vivo time-kil-curves for VRE under various linezolid concentrations are needed to describe the bacterial growth. LZD displays time- and concentration-dependent effects of killing with respect to VRE.

Vitamins E, C and placebo for 180 days. It was demonstrated that plasma levels of antioxidant strategies against the mentioned diseases has many causes, mainly the lack of antioxidants during aging. Moreover, human chronic diseases, as cardiovascular processes. Indeed a number of authors have shown a decrease in non-enzymatic antioxidative protection against vascular diseases, it remains to be studied in large controlled trials.

Financial support: Fapesp, CNPq, CAPES, and Marjan Indústria Farmacéutica.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
Antibacterial RNA Silencing

GOOD L

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Background: New strategies are needed to combat antibacterial resistance. Our strategy is to target bacterial genes at the RNA level as a flexible genetics tool and possibly a new strategy for drug development. RNA silencing (or antisense) technologies can effectively target essential gene transcripts to block bacterial growth, and provide a candidate for drugs to target pathogens.

Methods: We have developed short synthetic antisense peptide nucleic acids (PNA). Cell uptake is enhanced using attached cell penetrating peptides (CPPs). In a second strategy, we have developed stabilised, expressed antisense RNAs. Therefore, we are able to silence bacterial RNA using both synthetic and expressed antisense drugs. Both RNA silencing tools have been used to silence established and putative antimicrobial drug targets. The effects of RNA silencing were assessed using Northern analysis, RT-PCR and by monitoring bacterial growth.

Results: Synthetic antisense PNA kill bacteria when targeted to stringently-required essential genes in Escherichia coli, Staphylococcus aureus and Mycobacterium smegmatis. These synthetic RNA silencers accumulate in target cells and display a long post antibiotic effect in the absence of cell lysis. It is also possible to co-express gene silencers and E. coli by applying conscious sedation with midazolam.

Conclusions: Synthetic and expressed RNA silencers provide complementary new tools for antimicrobial drug development and drug mechanism of action studies. Interestingly, partial silencing, showed that established and putative antimicrobial drug targets differ significantly in their stringent requirement for growth. In other words, certain target genes are particularly sensitive to RNA silencing and may also provide more sensitive drug targets.

Background: For considerable decline in the mortality from breast cancer following systemic therapy, the biology of breast cancer remains poorly understood. Unfortunately, the routinely-used clinicopathologic variables fail to fully capture the biologic heterogeneity. As a result, many patients are overtreated whereas others may not receive the necessary therapy. Gene expression microarrays may provide more sophisticated information than conventional biomarkers in predicting disease outcome and response to a specific systemic therapy on an individual basis.

Methods: 1) To identify the molecular signature that predict pathologic complete response (pCR) to sequential treatment of paclitaxel, 5-fluorouracil, doxorubicin and cyclophosphamide (TAPIE) neoadjuvant chemotherapy, our group profiled 82 breast carcinomas and searched for the signature gene. Predicting accuracy of the signature was then validated on an independent set of 51 tumors. 2) To evaluate whether ER and HER2 status can be reliably measured from the comprehensive microarray data, we used gene expression data of 495 breast carcinomas to assess correlation between ER and HER-2 mRNA levels and clinical status of these genes (as determined by immunohistochemical and/or fluorescence in situ hybridization). Data from 165 fine-needle aspiration (FNA) samples was used to assess the correlation between ER and HER-2 mRNA levels and clinical status. Spearman’s correlation coefficients ranged from 0.62 to 0.77. The defined ER expression thresholds were assessed using parametric and non-parametric statistics.

Results: In two clinical studies among pediatric patients, we used needle aspiration combined with antibiotics as the initial treatment of suppurative cervical lymphadenitis and of perianal abscesses referred for incision and drainage. In both cases, a local anesthetic ointment was applied for 1 hour before the aspiration and incision procedure was performed using a 18-gauge needle. In some cases, a conscious sedation with midazolam was used.

Conclusions: Needle aspiration combined with antibiotics seems to be an effective and safe treatment of suppurative cervical lymphadenitis and of perianal abscesses. A complete regression of the nodes was obtained in all patients within 21 days, with no relapse or scar formation.

Table 1. Epididymal sperm count (x10

Control BM (160 mg/Kg) UPCH 0.01 g/Kg + BM UPCH 0.1 g/Kg+BM

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Control</th>
<th>BM (160 mg/Kg)</th>
<th>UPCH 0.01 g/Kg + BM</th>
<th>UPCH 0.1 g/Kg+BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>83.46±5.87</td>
<td>106.53±3.35</td>
<td>107.75±6.07</td>
<td>108.43±4.72</td>
</tr>
</tbody>
</table>

How did the MAGIC BULLETS drop the Knife out of the Surgeon’s Hand.

GORENSTEIN A,1,3 SIMEOKH E2,3, SERERO E1,3

1Department of Pediatric Surgery, Wolfson Medical Center, Holon, Israel; 2Pediatric Infectious/Immuno Unit, Wolfson Medical Center, Holon, Israel; 3Affiliated to Sacker Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Background: Surgical drainage has been considered the treatment of choice in several abscess formations. We demonstrated that surgical drainage may be avoided by the use of antibiotics in many cases. We have developed and tested several MAGIC BULLETS to treat cervical lymphadenopathy and of perianal abscesses referred for incision and drainage. In both cases, a local anesthetic ointment was applied for 1 hour before the aspiration and incision procedure was performed using a 18-gauge needle. In some cases, a conscious sedation with midazolam was used.

Conclusions: Needle aspiration combined with antibiotics seems to be an effective and safe treatment of suppurative cervical lymphadenitis and of perianal abscesses. A complete regression of the nodes was obtained in all patients within 21 days, with no relapse or scar formation.

Conclusions: Forty-seven infants (< 24 months of age) were treated by needle aspiration and antibiotics (gentamicin and metronidazole). A primary cure was achieved in 29 (61.7%) patients, and 16 (34%) children had an evolution toward a fistula in ano. Two infants had recurrent abscesses successfully treated by the same way.

Conclusions: Needle aspiration combined with antibiotics seems to be an effective and safe treatment of suppurative cervical lymphadenitis and of perianal abscesses. A complete regression of the nodes was obtained in all patients within 21 days, with no relapse or scar formation.

Conclusion: Needle aspiration combined with antibiotics seems to be an effective and safe treatment of suppurative cervical lymphadenitis and of perianal abscesses. This modality which does not require incision and drain removal, and does not lead to scar formation. We believe that our studies are additional steps for the continuous effort to replace invasive procedures by Magic Bullets.
Background: Targeting antigen (Ag) to Fc receptors (F-R) on Ag presenting cells enhances humoral and cellular immunity. Thus, we hypothesized that targeting inactivated F. lunanesis (Ff) to FcR intranasally (i.n.) would enhance protection against mucosal challenge. We examined: 1) the ability of anti-Ff monoclonal antibody (mAb) plus iFt, to enhance presentation of iFt Ag, 2) the ability of mAb-Ff administered i.n. to enhance protection against i.n. challenge, 3) the ability of an FcR-targeted subunit vaccine to protect against S. pneumoniae.

Methods: First, mouse macrophages and Ff Ag-specific T cells were combined with Ff or Ff+ plus anti-Ff mAb. Cells were incubated at 37°C, supernatants collected, and cytokine secretion measured. Second, mice were divided into three groups (5-6/group) and immunized i.n. with PBS, Ff, or mAb-Ff. Mice were immunized on day 0, boosted on day 21, challenged i.n. on day 35, and monitored 21 days for survival. Third, mice were divided into three groups consisting of wild-type (WT) mice immunized i.n. with PBS, WT mice immunized with S. pneumoniae Ag (PspA) in the form of anti-human FCyR1 (hFcγR1)-PapA subunit vaccine, or transgenic mice expressing hFcγRI transgenic versus WT mice, enhanced S. pneumoniae-specific IgA and IgG production, and protection against i.n. challenge with S. pneumoniae.

Results: Anti-Ff mAb plus iFt enhanced iFt presentation to Ag-specific T cells. When using mAb-Ff as an i.n. immunogen, increased protection (100%) was achieved compared to iFt alone (50%-65%). In addition, targeting PspA to hFc, delivered and tracked without arousing the suspicion of smugglers. The objective of our study was to develop a non-invasive MDCT (multi-detector computed tomography) screening method to detect cocaine-containing vessels that are hidden between uncontaminated ones in a shipment.

Conclusions: Studies were performed on three wine bottles containing cocaine solutions that were confiscated at the Swiss border. Reference values were obtained by scans of different sorts of commercially available wines and aqueous solutions of dissolved sugar. All bottles were scanned using MDCT, and data evaluation was performed by measuring the mean peak of Hounsfield units. To verify the method, simulated testing on twelve wine bottles including six contaminated bottles were performed.

Results: Using measurements of the mean peak of Hounsfield units, enables the detection of dissolved cocaine in wine bottles in a non-invasive and rapid fashion. Increasing opacity of the liquid corresponds well with the concentration of dissolved cocaine. Simulated testing showed, that it is possible to distinguish between cocaine-contaminated and uncontaminated wine bottles.

Conclusions: 1) The described method is an efficacious screening method to detect cocaine-containing bottles that are hidden between uncontaminated bottles in cargos. 2) The non-invasive examination of cargo allows a questionable delivery to be tracked without arousing the suspicion of the smugglers.
Mast cell.

GRAEVSKAYA E

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Mast cells are known to be among the most important effector immunocompetent cells participating in various biological responses. The description of the mast cell in connective tissue is ascribed to Ehrlich. Ehrlich made the important discovery that the mast cell stained well and specifically with certain dyes of the oiline family, the specific metachromatic staining reaction. Ehrlich also proposed the relation of mast cells with inflammation, blood vessels and neural tissue. Mast cell develop from progenitor cells that in turn arise from uncommitted hematopoietic stem cells in the bone marrow, they undergo terminal differentiation in tissues. It was determined that IgE molecules have a high affinity for specific receptors on mast cells and that the reaction of cell-bound IgE molecules with multivalent antigens or divalent anti-IgE antibody induces the release of a variety of chemical mediators from the cells, that testify a central role for the mast cell in immunological reactions. More recently the role of mast cells in inflammatory disease and host defense was established. Following activation, these cells express mediators such as histamine, leukotrienes and prostaglandins, as well as proteases, and many cytokines and chemokines, pivotal to the genesis of an inflammatory response. Mast cells have been shown to play roles in allergic inflammation, and more recently, they have been shown to modulate coagulation cascades, host defense and tissue remodeling. Although mast cells were discovered long ago they are certainly no less interesting today and their history is far from complete.

Delivery of specific targeted drugs into the cells by TAT-technology

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Background: The importance of drug delivery is pivotal in the wide area of pharmacological research and it has not been solved yet. The main goal of every drug delivery system is the delivery of a precise amount of a drug to the desired location in order to achieve the necessary drug concentration in the targeted organ for effective treatment. Proteins and peptides are useful research and therapeutic tools, however their applications are limited because delivery to the desired location is not easily achievable. Process of protein transduction, using TAT-technology, allows the delivery of drugs and genetic materials inside the cells. This process occurs in a rapid, concentration-dependent fashion that appears to be independent of receptors and transporters. It has a broad implications in experimental systems for regulating intracellular processes and has the potential to be used in the development of new therapeutic strategies for cancer, infectious diseases, and development of vaccines. Hyper proliferation of cancer cells is associated with deregulation of cell cycle progression, which is driven by the activities of CDKs. A key regulator of their activities is protein p27. It has significant role in cancer progression and antitumor drug response.

Results: To examine p27 as specific target molecule and its role in tumor cells apoptosis, transduction of TAT-p27, TAT-p27 and TAT-N1p27 was performed. It was shown that different forms of TAT-p27 protein can modulate the cell cycle of cultured cell lines and induced apoptosis, depending on the concentration and type of the cells. Also was shown that different signal transduction pathways were involved in induction of apoptosis.

Conclusions: Extracellular p27 could be used for induction of apoptosis in tumor cells. Protein transduction therefore could give an opportunity for delivering of drugs into cells with emphasis on specific target molecules.

The “antiinflammatory” 5-ASA, “immune-modulators” azathioprine, 6-MP, methotrexate & thalidomide and “immune-suppressants” Cyclosporine A, Rapamycin & Tacrolimus are all unsuspected “Magic Bullets” the inhibit M. avium subspecies paratuberculosis growth in culture

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Background: Without known mechanisms of action, “anti-inflammatories” (5-ASA), “immune-modulators” (azathioprine, 6-MP, methotrexate & thalidomide) and “immune-suppressants” (Cyclosporine A, Rapamycin & Tacrolimus) are used to treat a variety of non-malignant “dispatiche” and “autoimmune” diseases. M. avium subspecies paratuberculosis (MAP) causes a chronic wasting diarrheal disease in ruminants called Johne’s disease, that is evocative of human inflammatory bowel disease (IBD). MAP may be zoonotic. We hypothesized that, aside from their well-documented effects on eukaryotic cells, these agents may additionally be “Magic Bullets” that inhibit prokaryotes, particularly MAP. We herein report on the effects of these agents on MAP in culture.

Methods: We studied growth kinetics of four MAP strains (three human isolates, “Ben”, “Dominic” & UCF-4) a bovine MAP isolate ATCC 19698 and three mycobacterial controls (M. avium & BCG), using the radiometric 14 CO2 (Bactec®) system. Growth is quantified as arbitrary Growth Index (GI) units and inhibition as “percent decrease in cumulative GI” (%-GI).

Results: (Most published at www.PLoSOne.org Thalidomide data Unpublished) Our negative controls do not inhibit MAP. The test agents cause dose dependent inhibition of MAP. The most potent is methotrexate (85%-GI at 4µg/ml “Dominic”) followed by 6-MP, azathioprine, Cyclosporine A, Rapamycin, Tacrolimus and 5-ASA (40%- GI at 64µg/ ml ATCC 19698). Thalidomide comprises two components. Phthalimide causes no inhibition, whereas the piperidine 2,6-dione moiety inhibits MAP (46%- GI at 64µg/ml “Dominic”).

Conclusions: 1) We show in culture herefore-undescribed inhibition of MAP growth by a variety of agents that are used, simply because of empirical efficacy, to treat several “inflammatory” and “autoimmune” diseases. 2) These data show that these agents effect prokaryotic, in addition to eukaryotic cells. 3) We conclude that, unknowingly, the medical profession has been treating MAP infections since 1942, when Nanna Sivart introduced sulfasalazine. 4) We posit that MAP may be responsible for multiple “inflammatory” and “autoimmune” diseases that have been empirically treated with these agents.

Authors’ disclosure statement The author has submitted patents based on the hypotheses tested in these studies.
Cancer-testis antigens: effective molecules for developing successful immunotherapeutic strategies in the light of cancer complexity

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Background: Despite advances in our molecular knowledge, human cancer remains one of the major public health problems throughout the world. Although a number of immunotherapeutic (IT) and chemotherapeutic (CT) antigens have been suggested that they could be useful in the immunological treatment of cancer, the corresponding clinical studies lead us to redefine the need to establish new criteria for validating their applicability. Aims: 1) To discuss cancer as a complex dynamical disease. 2) To discuss CT antigens as effective molecules for developing immunotherapeutic strategies in the light of cancer complexity. Methods: This study investigates the immunolocalization of Spem protein 17, a label of CT antigen in multiple myeloma and carcinoma, in a panel of natural tissues of unrelated histologic origin and their tumoral counterparts. After deparaffination and rehydration, two-micrometers thick sections were placed in a bath with tris-buffered, 1% paraformaldehyde, then incubated with endogenous peroxidase blocking activity, and then treated with monoclonal primary antibodies raised against Sp17 (BD Biosciences). Mouse IgG, was used as negative control. This was followed by incubation with the DAKO Envision system. 3,3'-diaminobenzidine tetrahydrochloride was used as a chromogen to yield brown reaction products. Countstained slides were analyzed under a light microscope. Results: Sp17 was found in human germinal cells of the testis (except for spermatogonia), and in the citated epithelia of the respiratory airways and in both the male and female reproductive systems. Sp17 has also been recognized in ovarian inclusion cysts, melanophages of cutaneous melanomas, as well as in a variety of human primary tumor tissues, including a subset of esophagusneuroblastomas and a high number of pituitary adenomas. Conclusions: 1) Sp17 is more widely distributed in the human body than originally thought. 2) The expression of CT antigens is mainly studied at the level of gene expression and gene level measurement by RT-PCR analysis and the quantitative RT-PCR technology. However, the information provided by these approaches is limited by the fact that the phenomena observed at each level of anatomical organization have properties that do not exist at a lower or higher level. 3) A multidisciplinary system level approach, which takes into account the human being as a complex hierarchical system, provides a different way of investigating human cancer, thus promoting a more widely applicable of CT antigens for developing immunotherapeutic strategies.
Acetazolamide Inhibits Electrogenic Sodium Bicarbonate Flux through kNBC1. Molecular Mechanisms and Computer Simulations.

GROSS E

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Background: The HCO3−: Na+ cotransport stoichiometry of the electrogenic sodium bicarbonate cotransporter kNBC1 determines the reverse potential (Erev) and thus the net direction of transport of these ions through the cotransporter and thus across the cell membrane (i.e. efflux or influx). Phosphorylation of kNBC1-Ser(962) in the carboxy-terminus of kNBC1, by CAMP-protein kinase A (PKA), shifts the stoichiometry from 3:1 to 2:1. Downstream of Ser(962) in kNBC1 is a D986NDD motif. A homologous motif (D887ADD) in the protein kinase A (PKA), shifts the stoichiometry from 3:1 to 2:1. Downstream of kNBC1, a motif in this protein-protein interaction.

Methods: We used isothermal titration calorimetry to measure the binding constant of CAII to kNBC1 and Ussing chamber electrophysiology apparatus to measure the electrogenic flux of sodium and bicarbonate through the cotransporter.

Results: In isothermal titration calorimetry experiments, CAII was found to bind to wt kNBC1-C1 with a K(D) of 160 ± 10 nM. Acetazolamide inhibited the short-circuit current through the cotransporter by 66 ± 6 % when the latter operated in the 3:1 mode, but had no effect on the current in the 2:1 mode.

Conclusions: We propose a model in which CAII, when bound to kNBC1, builds a high local concentration of bicarbonate in the vicinity of the cotransporter’s anionic binding site. Phosphorylation of kNBC1 by PKA removes CAII and as a result lowers the local concentration of bicarbonate and shifts the stoichiometry to 2:1. This model is also supported by computer simulations with a six-state transport binding scheme and electric field modulated binding constants and membrane translocation steps.

Increased repolarize reserve as a new anti-arrhythmic principle

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Background: A healthy human heart will beat approximately 3,000,000,000 times during a normal lifespan without any disturbances. Any electrical deviation from this regular pattern is termed an arrhythmia. Arrhythmias can result in anything from minor palpitations to sudden cardiac death. A number of arrhythmias are due to a disturbed balance of the sodium and potassium ion concentration. In a number of ion channels, a disturbed balance of these ions can lead to an arrhythmia. The HERG channel is one of these ion channels and a disturbed balance of these ions is responsible for the occurrence of arrhythmias.

Methods: The experimental approach is translational. Patch-clamp experiments have been conducted applying native cardiomyocytes or by using heterologous expression systems in oocytes and mammalian cells of the HERG channel. In addition, ex vivo Langendorff experiments and in vivo studies in both conscious and anaesthetized animals have been conducted.

Results: A number of anti-arrhythmic properties was demonstrated for the HERG channel activator EKBM-669. EKBM-669, at low concentrations, increases the voltage-dependent inactivation of the HERG channel. EKBM-669 also reduces the incidence of extrasystolic and ventricular fibrillations in a rabbit heart failure model.

Conclusions: In conclusion we believe it is demonstrated that under certain circumstances, activation of the cardiac HERG channel can be a novel anti-arrhythmic principle.

Treatment of ovarian cancer cells with drug combinations targeting ErbB receptor tyrosine kinases and fatty acid synthase

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Background: The development of novel therapeutic strategies against ovarian cancer is still challenging. ErbB receptor tyrosine kinases (ErbBs) represent a promising target for new therapeutic approaches for the treatment of ovarian cancer. In ovarian cancer cells (OCC), ErbB2 (HER-2/neu) thus setting the stage for signal initiation. Importantly, both ErbB2 and ErbB3 are overexpressed in tumors including OCC and represent drugable targets. Recent data suggest a link between FAS and ErbB2 in breast cancer. In OCC, the relationship between FAS and ErbB2 is still elusive. Therefore, we examined the effect of FAS and ErbB2 on tumor growth and ErbB2 transcript levels, protein expression, and phosphorylation, but only weakly depresses mRNA levels. Strikingly, EKBM-669 also represses FAS mRNA and protein. CI-1033 also failed to affect EGFR and ErbB2 transcript levels, but compromised EGFR activity (but not EGFR protein expression) and ErbB2 protein expression and function. Generally, CI-1033 reduced ErbB2 function rather than ErbB2 protein expression. Moreover, CI-1033 correspondingly down-regulated FAS mRNA and protein. Our data indicate that ErbB and FAS pathways mutually interact with each other in OCC. Thus, interference with the FAS and the ErbB systems effectively ameliorates their oncogenic activities and may be exploited for OCC treatment. Supported by 'Initiative Krebsforschung', Vienna, Austria.
The PI3K/AKT pathway determines EGFR/HER/ErbB drug efficacy in breast cancer cells.

SHABBIR W, BRUNNER-KUBATH C, GRUSCH M, MARIAN B, WAGNER R, LÖTSCH D, ZIELINSKI C, GRUNTT W


ErbB transmembrane proteins belong to the family of tyrosine kinase receptors. Four members have been described: ErbB1 (EGFR), ErbB2, ErbB3, and ErbB4. ErbB1 and 2 are overexpressed/hyperactivated in many tumors, including ovarian and breast cancer. They stimulate carcinogenesis and malignant progression, and confer unfavorable prognosis. Clinical success has recently been obtained by targeting ErbB2 in ErbB2+ breast cancer. However, only 30% of ErbB2+ breast cancers respond to targeted ErbB2 blockade and most of the responders develop secondary resistance. The situation is even worse, when ovarian cancer is concerned. Unfortunately, predictive markers for assessing ErbB inhibitor sensitivity/resistance are still widely lacking. Using MIT assay and Western blotting we examined the effects of the novel irreversible ErbB inhibitor pertinib (EKB-569, Wyeth) on the growth activity and on ErBb-triggered signaling in 11 human breast and 11 human ovarian cancer cell lines. SKBR3 and T47D were identified as most sensitive and most resistant breast cancer cell lines, respectively. In contrast, the sensitivity of the ovarian cancer cell lines did not vary as much.

Dopamine transporter as the target carrier and illict and therapeutic drugs – PK/PD approaches to develop MAGIC BULLETS for cocaine abuse

GU H

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Background: Cocaine is a powerful psychostimulant and an addictive drug of abuse. There are three known high-affinity targets for cocaine: the dopamine transporter (DAT), the serotonin transporter (SERT), and the noradrenaline transporter (NET). Over decades of studies support the dopamine (DA) hypothesis that the blockade of DAT and the subsequent increase in extracellular DA primarily mediates cocaine reward and reinforces drug self-administration (SART, MIT, and AMPH). However, NET knockout mice still display the rewarding property of cocaine. These studies indicate that none of these transporters are required for the cocaine effects and led to the re-evaluation of the DA hypothesis and the proposal of redundant reward pathways. However, the knockouts have very significant adaptive changes during development to compensate for the complete absence of a critical protein, which might have altered how the animals respond to cocaine.

Methods: To study the role of DAT in cocaine reward, we have engineered a functional DAT knockout mice and two lines with targeted disruption of the DAT gene. The rats were then exposed to cocaine in the chamber for 1 hour. The DA metabolism in the brain was measured in vivo by a autoradiography technique.

Results: DAT-C0 mice, cocaine did not elevate extracellular DA in the nucleus accumbers (NAc), cocaine did not stimulate locomotor activity but suppressed it, and cocaine failed to produce reward as measured by conditioned place preference and by drug self-administration. In contrast, amphetamines, another psychostimulant, was able to stimulate locomotor activity and produce reward, indicating that the reward system functioned well in these mice. In addition, re-introducing wild-type DAT back into the brains of fully developed DAT-C0 mice restored the ability of cocaine to stimulate locomotor activity and produce conditioned place preference.

Conclusions: Our results indicate that cocaine blockade of DAT is required for the stimulating and rewarding effects of cocaine in mice with a functional DAT. While cocaine can induce reward in mice without DAT but it is through a mechanism different from those in normal mice. Therefore, under some abnormal conditions, possibly when the DA system is selectively effective, cocaine may produce reward by interacting with targets other than DAT. Furthermore, our results suggest that drugs that prevent cocaine binding to DAT should be further developed as potential treatments for cocaine addiction.

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Objectives: The present study focused on data regarding the current situation of drugs regulation in Pakistan, its developmental history and other related issues.

Introduction: Pakistan is committed to the goal of health for all, inspired by the principle of social equity. To achieve this, the government is taking all possible measures in general, and drugs in particular. National drug policy emphasizes to ensure the availability, efficacy, safety and quality of the essential drugs in Pakistan.

Methods: To develop a comprehensive overview of the laws and regulations prevailing in Pakistan, a review of relevant literature, national and international documentation, and interviews with experts were carried out.

Results: The present study provides a comprehensive overview of the current situation of drugs regulation in Pakistan. The legal and regulatory framework of Pakistan provides a systematic and organized framework for ensuring the availability, safety, and quality of essential drugs in the country.

Conclusions & Recommendations: The traditional system of medicines is not properly regulated and hence efforts are made to regulate it through law with a view to their rationalization, to improve standard and for the protection of the public from hazardous drugs. Comprehensive public information should be launched to enhance under standing and acceptance of the essential drugs concept by the health professionals. For the selection of essential drugs, the ease in availability of essential and genuine drugs should be ensured by the government.
Antileishmanial Efficacy of Amphotericin B bearing Emulsomes against Experimental Visceral Leishmaniasis

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Background: Human cytophrome P450 (CYPs) enzymes are oxidative enzymes responsible for the metabolic activation of many precarcinogens and participate in the activation and inactivation of anticancer drugs. Genetic differences of the detoxification system may cause an increase in susceptibility to environmentally induced bladder cancer. Genetic differences of the detoxification system may increase an increase in susceptibility to environmentally induced bladder cancer and prostate cancer. Aims: 1) To investigate the potential association between the cytophrome p450 1A2 (CYP1A2) gene polymorphisms and the risk of bladder cancer in a Turkish population. 2) To investigate the relationship of prostate cancer (PCA) with genetic polymorphism of 17-hydroxylase (CYP171)- (34 T/C) and CYP1A1 (T/C) genes in a Turkish population.

Methods: This study included 135 bladder cancer patients and 128 age and sex matched cancer-free controls, 146 prostate cancer patients, 136 benign prostatic hyperplasia patients, and 102 healthy individuals as controls. The polymorphisms were analyzed using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assay. Genotype and allele frequencies and their associations with bladder and prostate cancer risk, demographic factors, smoking status, and tumor stage were investigated.

Results: 1) There was no association between studied polymorphisms of CYP1A1 and CYP2D6 genes and bladder cancer risk. 2) No statistically significant association was observed between smoking status of the patients and any of the polymorphisms studied. 3) We have observed that there is statistically significant association between the smokers with the CYP1A2 CC genotype and the bladder cancer risk but not with the non-smoker subjects with CC genotype (OR=2.55; 95%CI, 1.030-6.316). 4) No association was observed between prostate cancer and 17 hydroxylase gene polymorphism. 5) There was also an association between 17 hydroxylase polymorphism and benign prostatic hyperplasia (P<.004).

Conclusions: These data demonstrate that cytophrome P450 enzymes polymorphisms studied may not be associated with bladder cancer and CAP population studied. In addition, the results suggest that the genotypes of CYP1A2 polymorphism may be associated with increased risk of bladder cancer in smokers.

Keywords: CYP450 polymorphisms, bladder cancer, prostate cancer

Ciprofloxacin Induces Oxidative Stress and Exerts Biphasic Cytotoxicity in Primary Culture of Rat Astrocytes and Human Fibroblast Cells.

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Background: Mechanism underlying adverse effects of ciprofloxacin (CPFX) is still not well known. Aims: The possible cytotoxic and oxidative stress inducing effects of CPFX were investigated on primary cultures of rat astrocytes and human fibroblasts.

Methods: The cultured cells were incubated with various concentrations of CPFX (0.5-300 mg/ml for astrocytes, n=4; 5-150 mg/ml for fibroblast cells, n=3), and cytotoxicity was determined only in fibroblast cells.

Results: 1) CPFX-induced cytotoxicity is related to oxidative stress. 2) The antioxidant N-acetylcyesteine and the glutathione precursor N-acetylcysteine protected astrocytes from CPFX-induced toxicity.

Conclusions: 1) CPFX-induced cytotoxicity is related to oxidative stress. 2) The hormetic-like biphasic effects of CPFX possibly resulted from the complex interdependent relationships between reactive oxygen species, cell proliferation, and cell viability.
Transient Activation of the Small GTPase Rap1 is Functionally Required for the Regulation of Breast Cancer Cell Motile Responses to IGF-I

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Background: The Ras family GTPase Rap1 is an essential regulator for adhesion receptors, actin dynamics, and cell migration. The mechanisms by which Rap1 controls motile responses in the epithelial cells remain unknown. In fact, current view is that activation of Rap1 tightens cell-cell junctions and thus may have anti-metastatic effects in cancer. Normal and cancer cells share common, and perhaps evolutionarily conserved, motility machinery activated by insulin-like growth factor I (IGF-I).

Methods: We used an in vivo tool, a fusion of enhanced green fluorescent protein (EGFP) to the Rap1 binding domain (RBD) of RalGDS that is recruited specifically to the sites of increased Rap1 activity. By confocal laser-scanning microscopy, we monitored dynamics of EGFP-RBD-RalGDS; by time lapse video microscopy we tracked localization of the EGFP-Rap1 molecules in live cancer cells. By image-based quantitative immunohistochemistry, we compared 23 normal/benign (N/B) and 32 invasive breast cancer (InvBC) surgical specimens.

Results: We report that in human breast cancer cells a rapid enhancement and a subsequent gradual decrease of Rap1 activity induced by IGF-I promote cell motile responses: breakdown of cell-cell contacts and formation of lamellipodia. This transient activation of Rap1 requires the kinase activity of the IGF-I receptor (IGF-IR) and receptor internalization. Time-lapse video microscopy in live cells confirmed a disappearance of EGFP-tagged WT-Rap1 and constitutively active V12Rap1 from cell-cell contacts. We also found accumulation of the active endogenous Rap1 in lamellipodia of IGF-I-stimulated cells, whereas selective blocking of IGF-I-induced Rap1 activation by over-expressed RapGAP restrained lamellipodia formation. Quantitative analysis of surgical specimens revealed significantly higher protein levels of Rap1 (p=6.23E-04) and IGF-IR (p=4.32E-07) in InvBC compared with N/B breast tissue.

Conclusions: 1) Presented data provide experimental evidence that transient activation of Rap1 by IGF-IR-mediated mechanism promotes cancer cell motile responses and that active Rap1 does not prevent cell-cell separation induced by IGF-I. 2) Screens of surgical specimens show significant over-expression of IGF-IR and its target Rap1 in infiltrating carcinoma of the breast.
Background: Septic arthritis is common in large animals and local therapy has proven to be favorable over systemic antibiotic treatment. Doxycycline (DOX) was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. 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DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. DOX was thought to encompass the spectrum against bacteria usually isolated from bovine and equine joints and to provide in addition a chondroprotective effect. Doxycycline was shown to be effective in the treatment of septic arthritis in both large and small animals. However, the use of this antibiotic for the treatment of septic arthritis in horses is limited due to the lack of appropriate approved formulations. A HPLC validated and improved method for the analysis of doxycycline in biological samples was developed and tested in animal models. The study revealed that doxycycline concentrations were significantly higher in serum than in synovial fluid, indicating that doxycycline penetrates synovial fluid effectively. This finding is important for the treatment of septic arthritis, as it suggests that doxycycline can reach the synovial joint in sufficient concentrations to achieve effective antimicrobial activity. The study also demonstrated that doxycycline concentrations were highest in synovial fluid obtained from joints with severe inflammation, indicating that inflammation may influence doxycycline penetration into synovial fluid. These findings have important implications for the treatment of septic arthritis, as they suggest that doxycycline may be an effective treatment for this condition, especially in cases of severe inflammation. However, further studies are needed to confirm the efficacy of doxycycline in the treatment of septic arthritis and to determine optimal dosing regimens. Overall, the results of this study suggest that doxycycline may be a promising treatment option for the management of septic arthritis in horses. Further research is needed to confirm these findings and to determine the optimal use of doxycycline in the treatment of this condition.
The insulin-like growth factor pathway- the key to overcoming resistance in cancer therapy?

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The insulin like growth factor (IGF) pathway is a complex signaling system that has importance in human growth and development. However, dysregulation of this system has been shown to be important in the proliferation and survival in many malignancies including lung, breast and ovarian cancer. Importantly, the IGF signaling has also been implicated as a mechanism of resistance to cytotoxic chemotherapy, hormonal therapy, radiation and biological therapy. Due to the potential of blocking these critical pathways of resistance, novel therapies targeting IGF-1R signaling have been developed and are currently being evaluated clinically. Initial investigations with agents targeting the IGF-1 receptor (IGF-1R) have proven to be very well tolerated and have demonstrated early signs of clinical activity. Among the most common adverse events is hypoglycemia. Recently, demonstration of IGF-1R inhibition having clinical improvement in response to chemotherapy was demonstrated in patients with non-small cell lung cancer. This enhancement of activity was most pronounced in the squamous cell subtype of non-small cell lung cancer, which has a relatively high expression level of IGF-1R. The preclinical and clinical activity of IGF-1R inhibitors will be reviewed. Accumulated data has suggested that crossstalk signaling between the IGF-1R and HER family of receptors (e.g., EGFR, HER2) is responsible for resistance to therapy targeting the individual pathways. Our group has recently demonstrated that the co-inhibition of IGF-1R and HER family of receptors has synergistic activity in multiple models in vitro, through blockade of “crosstalk” signaling. Based on these data and the goal of delivering the cancer killing agent to the target, we will be investigating whether crossstalk between the IGF-1R and HER2 is clinically apparent and important for resistance to HER2 targeted therapy. These early preclinical and clinical investigations have indicated that inhibition of IGF-1R signaling may be useful in overcoming resistance to many clinically important therapies.

The brain-blood barrier and its major transporters – a pharmacokinetic perspective on how to optimize drug delivery to the brain

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Background: Drug delivery to the brain is intricate due to the tight junctions and active influx transporters of the blood-brain barrier (BBB). Recently, also active efflux influx has been identified for some drugs. Thus, it would be possible to utilize efflux or influx transporters for the purpose of delivering less or more drug to the brain. Regarding increased brain drug delivery through active influx, very little is still to be achieved regarding the understanding and map active influx in relation to physico-chemical properties of drug candidates. Methods: In order to choose the optimal drug candidates for clinically relevant drug delivery to the brain, the methods used in early discovery and development need to be optimised on the relevant measures, i.e., the unbound, pharmacologically active drug concentrations. Unspecific binding in the brain parenchyma distorts the measures when total brain concentrations are utilised. The latter approach has for too long retarded successful development of drugs aiming at the brain. Also, focusing on the permeability (rate) seems to be of less importance for clinical relevance than focusing on the extent of drug delivery to the brain, although the former focus is by far the most common. The advantages with focusing on unbound concentrations include correlations of pharmacologically active concentration at the target site in to receptor binding properties or other pharmacodynamic actions. Results and Conclusions: We have therefore proposed that brain drug delivery be divided into three main aspects, the rate (P), the extent (Cl), and the affinity of the drug to brain tissue, described by the unbound volume of distribution in the brain (Vu.brain). In this way the unbound concentrations at the target site can be estimated from total brain concentrations and plasma concentrations after measuring fraction unbound, and be related to unbound plasma concentrations. This approach gives quantitative understanding on the role of active efflux or influx in vivo. Rapid methods to study these three aspects are needed in early drug discovery and development. At the same time, in vivo knowledge is needed to validate the methods. We are presently focusing on optimising methods for this purpose.

Rho-kinase Inhibitors Augment the Inhibitory Effect of Anesthetic Agents on Rat Airway Smooth Muscle Contraction

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Background: Most anesthetic agents relax airway smooth muscle (ASM). ASM contraction is caused by both increasing intracellular Ca2+ (Ca2+3) and sensitivity. The preclinical and clinical activity of IGF-1R inhibitors has been demonstrated in cancer therapy. Inhibition of RhoA and Rho kinase (ROCK) play important roles in regulating Ca2+ sensitivity. In this study, we investigated the effects of selective ROCK inhibitors on ASM contraction and the influence of ROCK inhibitors on anesthetic-induced relaxation in ASM to test the hypothesis; although both anesthetics and ROCK inhibitors relax ASM independently, anesthetic-induced relaxation would be enhanced by addition of a low concentration ROCK inhibitor. Methods: This study included 50 male Wistar rats (6 weeks, weight 180 – 220g). Ring strips from intrapulmonary bronchi were placed in 400-µL organ baths containing Krebs–Henseleit solution. After obtaining stable contraction with 30 µM acetylcholine (ACh), isometric forces were measured with the following protocols: A) Y-27632 (0.01 – 300 µM), fasudil (0.01 – 100 µM) and H-1152 (0.01 - 100 µM) were cumulatively applied. (C) Y-27632 significantly shifted the concentration-response curves to the left (p<0.01, ANOVA, followed by Bonferroni’s test (p<0.05 was considered significant). Results: (A) All ROCK inhibitors, especially H-1152, decreased ASM concentration-dependent relaxation (n=6 each). (B) 0.03 µM Y-27632 and fasudil did not affect the relaxation by propofol, while 0.1 µM both agents significantly shifted concentration-response curves to the left (p<0.05 (Y-27632), p<0.03 (Fasudil)). (n=6 each). H-1152 (0.03 and 0.1 µM) significantly shifted the concentration-response curve to the left (p<0.001). (n=6 each) (C) Y-27632 significantly shifted the concentration-response curve for isoflurane to the left. (P<0.001) (n=6). Conclusions: 1) ROCK inhibitors augment anesthetics-induced relaxation of rat ASM. 2) Combined use of ROCK inhibitor and anesthetics may be useful for anesthetic managements and the treatment of asthmatic patients.
Polyethylene Glycol Gold Coated Nanoparticles for the Enhancement of the Efficacy of a Specific Nutrient Synergy

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**Background:** The cyclopolypeptide CAM741, a derivative of the natural compound HuI-2793, is a potent and selective inhibitor of vascular cell adhesion molecule 1 (VCAM1) expression on endothelial cells. CAM741, besides its powerful antiproliferative efficacy, is a potent and selective inhibitor of vascular cell adhesion molecule 1 (VCAM1), expression on endothelial cells.

**Results:** Extensive studies on the mechanism of action led to the identification of the signal peptide of VCAM1 as target of the compound action. The inhibitory effect of the compound takes place at the signal peptide-dependent process of cotranslational translocation. While CAM741 does not inhibit targeting of the VCAM1 nascent chains to the Sec61 translocon, it inhibits the translocation of the polypeptide chains to the luminal side of the endoplasmic reticulum. Chemical crosslinking further demonstrated that targeted VCAM1 nascent chains are differently associated with the translocon in the presence of compound, and that also the signal peptide itself shows altered positioning within the Sec61 translocon. During a search for other CAM741-sensitive signal peptides, that of vascular endothelial growth factor-A (VEGF) was identified as another target of the compound. Although both signal peptides are sensitive to CAM741, they do not differ in their sensitivity towards the compound. Sensitivity to CAM741 is a key feature of the signal peptides that are sensitive to CAM741.

**Conclusions:** Our current model of the compound action depicts that the efficiency of signal peptide binding to the Sec61 translocon at least contributes to the inhibitory action of CAM741 and that the compound competes with the incoming signal peptide for translocation binding. Further investigations on the mechanism of translocation inhibition and the search for other sensitive signal peptides should help to dissect the mode of action and to understand the process of cotranslational translocation driven by signal peptides. These studies provided the first proof-of-principle that the process of cotranslational translocation can be inhibited in a signal peptide-specific manner without affecting the overall translocation process of other polypeptides.

Selective inhibition of signal peptide-dependent cotranslational translocation by the cyclopolypeptide CAM741

HARANTE H, OBERHAUSER B, DE VRIES JE, LINDLEY IJD
Novartis Institutes for BioMedical Research, A-1235 Vienna, Austria

**Background:** The cyclopolypeptide CAM741, a derivative of the natural compound HuI-2793, is a potent and selective inhibitor of vascular cell adhesion molecule 1 (VCAM1) expression on endothelial cells.

**Results:** Extensive studies on the mechanism of action led to the identification of the signal peptide of VCAM1 as target of the compound action. The inhibitory effect of the compound takes place at the signal peptide-dependent process of cotranslational translocation. While CAM741 does not inhibit targeting of the VCAM1 nascent chains to the Sec61 translocon, it inhibits the translocation of the polypeptide chains to the luminal side of the endoplasmic reticulum. Chemical crosslinking further demonstrated that targeted VCAM1 nascent chains are differently associated with the translocon in the presence of compound, and that also the signal peptide itself shows altered positioning within the Sec61 translocon. During a search for other CAM741-sensitive signal peptides, that of vascular endothelial growth factor-A (VEGF) was identified as another target of the compound. Although both signal peptides are sensitive to CAM741, they do not differ in their sensitivity towards the compound. Sensitivity to CAM741 is a key feature of the signal peptides that are sensitive to CAM741.

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Proton Transport Inhibitors (PTI) as Selective Anticancer Agents

HARGUNDEY SA, ARRANZ JL, WAHL ML, RESKIN SJ, CRIVE G
1Institute of Clinical Biology and Metabolism, c) Postas 13 - 01004 Vitoria, Spain; 2Department of Pathology, DUUM 3712, Duke University, Durham, NC 27710, USA; 3Department of General and Environmental Physiology, University of Bari, 70126 Bari, Italy; 4Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of the Basque Country, c) Paseo de las Universidades 7 - 01008 Vitoria, Spain.

CONFERENCE OUTLINE AND SUMMARY

“We can only cure what we can understand first”. Otto Warburg

A proton (H+)-related mechanism underlying the initiation and progression of the neoplastic process has been recently described by different research groups. Regardless of their origin and genetic background, all cancer cells and tissues have a pivotal energetic and homeostatic disturbance of their metabolism that is completely different from all normal tissues: an aberrant regulation of hydrogen ion dynamics leading to an intracellular pH to extracellular pH (H+ or pH) gradient reversal of cancer cells and tissues, that leads to an interstitial acid microenvironment secondary to an initial, specific and epiphenomenic intracellular alkalosis. This specific abnormality is increasingly considered as one of the most sensitive and differential hallmarks of cancer. This approach, which focuses on the relationships among the intracellular and the extracellular dynamics of the hydrogen ion permits the creation of a unifying view of several of the most important fields of cancer research, from epilphenogies, cancer cell metabolism and neovascularisation, to multiple drug resistance (MDR), selective apoptosis, the metastatic process, cancer chemotherapy and even the spontaneous regression of cancer (SRC). The integral and rational perspective behind these findings is likely to open new pathways towards the development of more selective and less toxic therapeutic measures for all malignant diseases. New therapeutic approaches are advanced.

The Development of Tumor-Inhibiting Metal Complexes: (Multinuclear) Metal Complexes and Mode-of-Action Studies

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1University of Vienna, Vienna, Austria; 2EPFL, Lausanne, Switzerland.

Background: Platinum-based drugs are widely used in the therapy of cancer although being only active in a limited number of tumors and often exhibiting severe side effects. Ruthenium compounds are the best examples of non-platinum anticancer agents, in particular with KP1019 and NAMI-A, two ruthenium(II) compounds, undergoing clinical trials. In recent years, organometallic ruthenium(II)-arene complexes moved into the focus of interest and compounds with activity against primary tumors and antimetastatic activity were reported.

Methods: The synthesis, (bio)analytical characterization and in vitro anticancer studies of dinuclear (η5-p-cymene)(thiurum)3 complexes with varying spacer length are reported. The compounds were characterized by NMR spectroscopy and ESI mass spectrometry, and the molecular structure of 1,6-bis(chlorido)- (η5-CO2H2)(η5-p-cymene)(thiurum)3 complexes with varying spacer length were reported. The compounds were characterized by NMR spectroscopy and ESI mass spectrometry, and the molecular structure of 1,6-bis(chlorido)-(η5-CO2H2)(η5-p-cymene)(thiurum)3 complexes with varying spacer length were reported. The compounds were characterized by NMR spectroscopy and ESI mass spectrometry, and the molecular structure of 1,6-bis(chlorido)-(η5-CO2H2)(η5-p-cymene)(thiurum)3 complexes with varying spacer length were reported.

Results: The coupling of two (η5-p-cymene)(thiurum)3 moieties via alkyl-pyridine spacers (alkyl = propane, hexane, dodecan) resulted in compounds 1–3 with IC50 values in the low micromolar range against the human tumor cell lines A5780 and SW480, whereas the mononuclear analogue 4 is not active. The anticancer activity was found to be dependent of the spacer-length (see Table), which also influences the lipophilicity of the complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 /µM</th>
<th>A5780</th>
<th>SW480</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (C3)</td>
<td>26 ± 2</td>
<td>62 ± 14</td>
<td></td>
</tr>
<tr>
<td>2 (C6)</td>
<td>30 ± 6</td>
<td>26 ± 8</td>
<td></td>
</tr>
<tr>
<td>3 (C12)</td>
<td>1.5 ± 0.3</td>
<td>0.29 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: Ru(II)-arene metallo-drugs with spacer-length-dependent activity in human tumor cell lines were developed. Notably, not only is an additive effect of the analogous mononuclear complexes observed but a synergistic effect in two cell lines was present – in the SW480 cells being of extraordinary dimension for Ru compounds.

Hierarchy of Immune Responses behind the Blood-Brain-Barrier (BBB) in the Normal Brain: Implications for the development of CNS Diseases and Treatment

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Background: Interactions between the brain and immune system are hierarchical and highly regulated. We have shown that antigens microinfused into the rodent brain reach cervical lymph nodes by outflow pathways for brain fluids along cranial nerves and stimulate a specific response in nodes within days. Tumor cells in the brain activate cytotoxic T lymphocytes (CTL) but do not destroy the brain tumor due to suppression of CTL activity by TGF-β in cerebrospinal fluid. Delayed type hypersensitivity response to microinfused protein fails to develop while the same dose elicits a robust serum antibody (Ab) response and intrathecal Ab synthesis, without inflammation, that persists for months. Aim: To determine if the presence of Abs behind the BBB can alter brain function if they cross-react with brain antigens.

Methods: Multiple studies (I-IV) were done using a rodent model with normal BBB function. A. cannula was stereotaxically implanted into rat brain and 7 days later, dilute sera or immunoglobulins (Igs) with anti-neuronal activity were microinfused (Alzet® pump) through the cannula. Controls received normal sera or lgs. In study I, sera or lgs from children with Tourette Syndrome (TS) were microinfused bilaterally into caudate nucleus (CN) and rats were assessed for stereotypies and Ig binding to neurons. In studies II-IV, cannula placement was into the region of the subthalamic nucleus (STN) and the turning response to s.c. injection of apomorphine was measured following unilateral microinfusion of: sera from children with Sydenham’s chorea (SC), Study II; rat anti-sera against rheumatogenic streptococci (Strep), which contains brain-cross-reactive epitopes, Study III; and rabbit-anti-lgs against a peptide of oligodendroglialocyte myelin glycoprotein, Study IV.

Results: Rats microinfused with TS sera or lgs (I) had significant changes in spontaneous behavior (licking and vocalization p < 0.04) and TS-Igs were bound to neurons in CN. Rats receiving a microinfusion of anti-neuronal Abs into STN (II- IV) had significantly greater ipsilateral rotational turning behavior in response to the bioassay compared to control rats. The altered turning response persisted for weeks. In Study III, lgs were bound to neurons within the ventral striatum, an area linked to movement. Conclusion: Antibodies capable of binding brain antigens can affect brain function if they are placed behind the BBB. Affinity of Ehrlich’s magic bullets (Ab) and the brain milieu, supportive of Abs synthesis, may be a double-edged sword if synthesized Abs recognize brain epitopes.

Orally Ingested Lactoferrin and Glycine Display in vivo Synergistic Anti-Inflammatory Activity

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Background: There is a growing awareness of the interaction of food constituents with the immune system. The developed metabolic effects of two of these nutritional components, i.e. glycine and lactoferrin, were studied with the immune system. The present studies aim to evaluate immunomodulatory effects of two of these nutritional components, i.e. glycine and lactoferrin.

Methods: Mice orally supplemented with glycine, lactoferrin or a combination, were injected intradermally in the ear, with zymosan. Ear swelling (as a measure for inflammation) and the number of TNF-α producing spleen cells were analysed. In a collagen induced arthritis (CIA) model mice were orally supplemented with a combination of glycine and lactoferrin starting after the second collagen booster. Arthritis development was scored and the pro-inflammatory cytokine levels in the serum were detected.

Results: Glycine and lactoferrin were able to decrease the zymosan induced pro-inflammatory response locally (increased ear swelling) as well as systemically (reduced number of TNF-α producing spleen cells). Glycine effects (20, 50 and 100 mg/mouse/day) were concentration dependent whereas for lactoferrin only the lowest doses (0.1 and 1 mg/mouse/day) inhibited the inflammatory response significantly. Surprisingly higher doses of lactoferrin (5 and 25 mg/mouse/day) failed to influence the inflammatory reaction. A combination of both nutrients (lactoferrin 0.1mg/mouse/day in combination with glycine 20 or 50 mg/mouse/day) inhibited the zymosan induced ear swelling synergistically. In the CIA model the combination of glycine and lactoferrin (lactoferrin 0.1mg/mouse/day with glycine 20 mg/mouse/day) was able to inhibit arthritis development and decrease the level of pro-inflammatory cytokines in the rats.

Conclusions: The present data indicate that the glycine-lactoferrin concept might offer in the near future a powerful nutritional way in modulating chronic inflammatory diseases.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts  Page A-119
Cancer stem cells - towards a new generation of antineoplastic drugs

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Background: There is evidence accumulating that tumour growth is perpetuated by a rare subpopulation of tumour cells, the cancer stem cells. It is increasingly clear that these cells are causally implicated in therapy resistance, tumour relapse and resulting treatment failure. The typical cancer stem cells with self-renewing, intrinsically chemoresistant properties are a consequence of drug efflux and detoxification activities and display a high level of DNA-repair activity.

Methods: We have performed a literature review of publications directly relating cancer therapy resistance and cancer stem cells. We have developed a method of visualization of cancer stem cells in living cell cultures, based on isolation of doxorubicin resistant bladder carcinoma cell line and a doxorubicine – reporter gene construct.

Results and Conclusions: Direct relationship between cancer chemoresistance and cancer stem cells was obtained for a range of tumours, including colon cancer, glioblastoma, hepatocellular carcinoma, endometrial carcinoma, nasopharyngeal carcinoma, ovarian cancer, lung cancer, as well as leukaemia. We are able to visualize naturally chemoresistant cells in a bladder carcinoma cell line and we can show that these intrinsically chemoresistant cells are uniquely clonogenic and responsible for growth restoration after the experimental doxorubicin treatment, thus fulfilling the essential criteria for cancer stem cells. Our method of visualization of cancer stem cells might facilitate identification of therapeutic compound specifically targeting cancer stem cells, which might be an attractive way for future cancer chemotherapy. Targeting normal tissue stem cells by such a stem cell – directed therapy would represent an essential limitation of these approaches of anticancer therapy.

Combination with hyperthermia and radiation contributes to the magic of cisplatin in cancer treatment.

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Background: It has been shown that hyperthermia/hyperthermia kills preferentially tumor cells, but not to an extent that it can be used alone. Combination with other treatment modalities is apparent very favourable as many preclinical studies have shown that hyperthermia improves cell killing by radiation and chemotherapy. Promising chemotherapeutic agents in combination with hyperthermia include taxanes and derivatives, gemcitabine, melphalan, cyclophosphamide, BCNU, bleomycin, vincristine and melphalan.

With the clinical application of hyperthermia temperatures in the range of 40 to 45 °C are used. A temperature of 45 °C or higher is reached either by hyperthermia alone or in combination with radiation and chemotherapy. A variety of mechanisms have been described as to how hyperthermia may enhance the effects of radiation and chemotherapy. As a result of this, hyperthermia is currently investigated as an experimental therapy in the clinic.

Methods: The effect of incubation of cells for 1h at 41 °C on the accumulation of Rad51, Mre11 and Mcd1 (proteins involved in DNA DSB repair) in irradiated radia

Results: Incubation of cells at 41 °C leads to a temporary inhibition of Rad51 accumulation in IRF and HR efficacy. This inhibition of HR was accompanied by an increase in the number of chromosomal translocations which could be prevented by incubation of cells with high (> 10µM) concentrations of cisplatin. Our results suggest that in G2 phase of the cell cycle the decision which pathway to use for repair of IR-induced DSBs is made early after damage induction. After inhibition of HR, DSB repair might be shifted to the error-prone NHEJ pathway resulting in the rapid formation of chromosomal translocations.

Conclusions: These preclinical results point to favourable effects when three modalities are combined, also at relatively low hyperthermic temperatures. Three modality treatment is currently investigated as an experimental therapy in the clinic.
Gold activates mast cells through L-type calcium channels.

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Background: Xeroderma pigmentosum, gold and silver have been shown to induce allergic and autoimmune in genetically susceptible humans and/or experimental animals. Mast cells are implicated to play a role in the development of these adverse immunological reactions. Recently, it is suggested that both allergenic and autoimmune mechanisms are involved in autoimmune diseases, including multiple sclerosis, a metal-associated disease. We have previously shown that L-type calcium channels (LTCCs) play a critical role in regulating mast cell activation. Here we demonstrate that gold activates mast cells through LTCCs via the production of H2O2.

Methods: Degranulation was determined by β-hexosaminidase and leucotriene C4 (LTC4) secretion was measured using an enzyme-linked immunosorbent assay (ELISA). The production of intracellular H2O2 was measured using DCFH-DA by flow cytometry. The cytotoxic calcium concentration ([Ca++]i) was measured using the Fluo3/AM.

Results: Au(1+) at concentrations of ranging from 10 μM to 100 μM dose-dependently induced degranulation and LTC4 secretion with a minimal cytotoxicity. In parallel, Au(1+) stimulated the production of intracellular H2O2 and scavenging the oxidant by the glutathione peroxidase mimetic esbenol blocked [Ca++]i increase, degranulation, and LTC4 secretion. Subsequent studies revealed that Au(1+) stimulated LTCC activity, which was activated by H2O2. The effects of Au(1+) were partially similar to those of Hg(2+) and As(3+). Further investigations on the role for LTCCs using LTCC gene silencing are underway.

Conclusions: Au(1+) appears to utilize a unique ROS- and LTCC-dependent mechanism which is partially overlapping with those activated by Hg(2+) and As(3+). This finding may explain the fact that the three xeroderma pigmentosum metals induce autoimmunity by similar but not identical mechanisms.

Increase of fibrin network porosity and the consequent fibrinolysis as an anticoagulant effect of acetylsalicylic acid

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Karolinska Institutet: 1Dept of Clinical Sciences, Danderyds Hospital; 2Dept of Molecular Medicine & Surgery/Coagulation Research, Sweden, Stockholm

In patients with stable angina pectoris, the fibrin network permeability shown as scavenging the oxidant by the glutathione peroxidase mimetic esbenol blocked [Ca++]i increase, degranulation, and LTC4 secretion. The cytotoxic calcium concentration ([Ca++]i) was measured using the Fluo3/AM.

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Cardiotoxicity Plagues Bupivacaine

HEAVNER JE
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Background: Bupivacaine was introduced into clinical practice as a local anesthetic in 1963. Not until 1979 did serious concerns about bupivacaine-induced cardiotoxicity surface following fatal-maternal deaths when bupivacaine was used for epidural analgesia during childbirth. This led to refinement in assumptions about structure-activity relationships for local anesthetics and investigations that furthered understanding of the biological actions of this class of drugs.

Methods: Literature reports of clinical, translational and fundamental investigations of the cardiotoxic effects of bupivacaine were reviewed. Key observations were summarized.

Results: Resuscitation from bupivacaine-induced cardiovascular collapse is difficult. The ratio of bupivacaine central nervous system toxic dose to cardiovascular toxic dose is narrower than that for other local anesthetics. Kinetic difference in binding to sodium channel sites influences relative difficulty in resuscitation from bupivacaine cardiotoxicity vs other local anesthetics. The kinetic differences also influence pro and anti arrhythmic activity. Bupivacaine differs from other local anesthetics with respect to the spectrum of effects it has on voltage gated ion channels and on ligand gated receptor signaling. The S isomer of bupivacaine is more potent and less toxic than the R isomer or racemic mixture.

Conclusion: After being considered a "silver bullet" for producing long lasting local or regional anesthesia for 16 years, bupivacaine is now plagued by cardiotoxic effects that sets it apart from other clinically used local anesthetics.
Biopharmaceuticals in plants: toward the next century of medicine.1

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Plants present a novel means by which large quantities of vaccine and therapeutic proteins can be produced in a safe and cost-effective manner. Biopharmaceuticals produced in plants are easy to store, require fewer timely and expensive purification steps, and lack the containment risks associated with proteins produced in animal or bacterial expression systems. Over the past decade, much progress has been made with respect to the development of vaccines, antibodies and other therapeutic proteins. This presentation outlines the steps involved in the generation of transgenic plants, the engineering of plant virus expression vectors for transient expression of vaccine proteins and other therapeutics in plant tissue, and the advantages of this technology over the use of conventional transgenic plants. An investigation into the basis of mucosal immunity using plant-based oral vaccines is addressed. The scale-up of plant-derived vaccine proteins in entire crops or in large batch cell suspension cultures is covered, as is the development of clinical trials utilizing plant-derived biopharmaceutical proteins. Risks involved and biosafety concerns regarding plant-derived biopharmaceuticals are investigated. The presentation will conclude with a discussion of the future of plant-based vaccines and other therapeutic proteins in human and veterinary medicine with respect to commercial viability and as a tool to improve global public health.

Matching the Individual Patient to the Results of Large Clinical Trials and Testing the Null Hypothesis Using Fuzzy Theory

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2Medical College of Wisconsin, Milwaukee, WI, USA

Background: Translation of results of large clinical trials to the individual patient who always is to some degree and in many ways unlike any patient in that trial is impossible.

Methods: We hypothesized that 1) fuzzy measures of substratum and entrophy precisely match any individual patient to the average patient of any large clinical trial, and 2) that fuzzy substratum and entrepy can be used in the experimental setting to precisely measure the difference between patient states at different points in time and between groups of patients. Fuzzy entropy is the measure of the sameness or non-difference between elements and is valued in the unit interval of zero to one. Elements of a fuzzy set are each valued in the unit interval. One patient can be represented by a fuzzy set of elements of biophysiological or other clinical interest. The unit interval value of any element is determined by laboratory patient can be represented by a fuzzy set of elements of biophysiological or other.

Results: Fuzzy substratum measures the degree to which fuzzy set A belongs to fuzzy set B. The degree to which fuzzy set A belongs to B and A to B is a measure of the similarity between fuzzy sets A and B. The measure of difference between conditions, unknown or unmeasured variables, of two fuzzy sets A and B is measured by K, derived from fuzzy substratum, and when accounted for gives a true comparison of fuzzy sets A and B. Following one patient from fuzzy state A to B that patient has changed from one point in time to another. The substratum measure compares these states. In the experimental setting, this measure is applied to each patient of control and experimental groups. When testing the null hypothesis, the fuzzy entropy measure is the measure of “no difference” between control and experimental patient (s). A fuzzy entropy measure of 1 confirms the null hypothesis. A measure of match between a patient at the bedside and the average patient of any clinical trial is the measure of similarity of those patients accounting for the difference in their context. This measure falls within the unit interval and can be multiplied by the risk reduction, or other statistic of the trial to read a predictive value for that unique patient.

Conclusion: Fuzzy measures allow statistical results of large clinical trials to be exactly matched to the unique individual patient at the bedside.

Aciclovir- a Nearly Atoxic Antiviral Drug with Severe Neurotoxic Side Effects- a Retrospective Review of 280 Cases and the Importance of Analyzing the Aciclovir Metabolite CMMG

HELLDÉN A
Laboratory Medicine, Stockholm, Sweden

Background: Acyclovir (ACV) and its prodrug valaciclovir (VACV) is an effective agent against herpes simplex (HSV) and varicella zoster virus (VZV) infections. It is regarded as a nearly non-toxic drug. However, aciclovir-induced neurotoxicity (AIN) has been reported, predominantly in patients with renal impairment. AIN may be difficult to distinguish from the CNS-infection itself. We have earlier shown that the metabolite of aciclovir, 9-carboxymethoxymethylguanine (CMMG), is inconsistently increased and above 10 µmol in serum (S) in AIN. This study elucidated if AIN and HSV encephalitis symptoms could be separated from each other and if measurement of CMMG could be a marker of AIN.

Methods: Published case reports on suspected AIN, cases reported to the Swedish adverse drug reactions database “SWEDIS”, and cases investigated at the Karolinska University Hospital were reviewed. Type and frequency of ACV side-effects, renal function at the start of (V)ACV treatment, and serum concentrations of ACV and CMMG were studied. Three published reports on herpes encephalitis and the type and frequency of the initial symptoms were also included.

Results: 280 patients with AIN were found. Sixty-five percent were treated due to VZV, 18% due to HSV and 17% due to other causes. Chronic renal failure was present in 168 (60%) and acute renal failure or increasing S-creatinine in fifty-six (20%). The most frequent CNS-symptoms were confusion/disorientation, hallucinations and fatigue. ACV (N=124) and CMMG (N=77) concentrations were (mean ± SD) 44.4 ± 55.0 µmol/L and 38.3 ± 38.6 µmol/L, respectively. Sixty-two of the 77 AIN patients (81%) with S-CMMG had a concentration above 10 µmol/L. Patients with HSV encephalitis (N=197) presented with altered consciousness, fever, personality changes, confusion/disorientation and seizures.

Conclusions: 1. AIN is not uncommon and probably underdiagnosed, especially as the initial symptoms from CNS-symptoms from herpes encephalitis or AIN. The method is already in use in several Swedish hospitals with promising results.

All abstracts are listed in alphabetical order of the presenting author.
Glucagon-like Peptide-1: Broadening the Incretin Concept to Involve Gut Motility; a New Target for Treatment of IBS

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Background: Glucagon-like peptide-1 (GLP-1), an incretin, that is a peptide hormone released from the gut and enhances insulin release from the pancreatic islets after food intake. In addition to that GLP-1 is also considered to have smooth muscle relaxing properties and causes a profound slowing of gastric emptying rate, making it ideal for the treatment of diabetes type 2. We have previously shown in rat and man that GLP-1 also inhibits small bowel motility. This has led further to evaluate this endogenous peptide for use in initable bowel syndrome (IBS), where symptoms most likely are due to pressure build-up in the gut lumen. Therapies are needed for IBS as the impact of this disease is considerable in terms of individual suffering and economic cost. By causing relaxation of gastrointestinal smooth muscle, it is thought that the GLP-1 analog, ROSE-010, should be capable of alleviating pressure build-up in the gut, reducing IBS-associated pain.

Methods: This phase IA, prospective, randomized, cross-over, double-blind, placebo-controlled, multicenter study enrolled 166 subjects with irritable bowel syndrome. Subjects received single subcutaneous injections at each clinic visit (placebo, 100 µg or 300 µg ROSE-010) within 1 hour of a pain attack. The primary efficacy endpoint was total pain relief response as evaluated by visual analog scale. A clear pain with a reduction of pain of more than 55% in two hours. Secondary endpoints reflected different aspects of IBS pain relief such as meaningful and cumulative pain relief.

Results: ROSE-010 was superior to placebo (response rate 24.2% in total pain relief response compared with 12.0% for placebo [P=0.0053]) with regards to the primary endpoint. ROSE-010 was also more efficacious than placebo as assessed by maximum total pain relief, area under curve for pain relief and summed pain intensity, intensity of pain and summed pain intensity. In addition, the effect of ROSE-010 was also associated with a clear relationship to the intake of food. No hypoglycemia or other safety concerns were identified for ROSE-010.

Conclusions: The GLP-1 analog, ROSE-010 (100 µg or 300 µg), was superior to placebo in relieving acute abdominal pain in irritable bowel syndrome. ROSE-010 was safe and well tolerated. These results show that ROSE-010 is a potential treatment for pain in subjects with IBS.

Authors disclosure statement: HS and JK were supported by the sponsor during the study. Other authors have no disclosures.

Pharmacokinetic interactions of drugs and fruit juices with carbamazepine in Rat

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Background: Carbamazepine is an antiepileptic agent metabolized by CYP3A; hence CYP3A inhibitors or substrates may interrupt with carbamazepine. There are however limited data on how fruit juices, and indeed fruit juices with carbamazepine pharmacokinetics in rats were evaluated. Simvastatin (10 mg/kg) or 2 ml of juice or placebo were administrated. On the other hand, the elimination half-life of GLC was performed by high performance liquid chromatography. Therefore, in this study, we investigated the CYP3A-mediated drug interaction in rat.

Methods: Male Wistar rats, weighing 280 to 300 g, were used in the study. The effects of simvastatin, pomegranate juice, and star fruit juice on carbamazepine pharmacokinetics in rats were evaluated. Simvastatin (10 mg/kg) or 2 ml of juice or water was orally administered to rats (N = 6). Carbamazepine at a dose of 50 mg/kg was orally administered through gastric intubation at 1 h after the previous drug administration. Experimental samples (approximately 5 ml) were collected at 10, 30, 60, 90, and 120 min after oral administration of carbamazepine. Analysis of carbamazepine and carbamazepine 10.11-epoxide was performed by high performance liquid chromatography.

Results: In contrast with blood, the area under the concentration-time curve (AUC) of carbamazepine was lower in the group treated with pomegranate juice or star fruit juice, than that of the control group. The AUC of carbamazepine was determined by the injection of pomegranate juice. Carbamazepine AUC was increased approximately 1.3-fold when simvastatin or star fruit juice was administrated in pharmacokinetic parameters are shown in the table (average SEM; *p<0.05).

<table>
<thead>
<tr>
<th>parameter</th>
<th>control</th>
<th>simvastatin</th>
<th>pomegranate</th>
<th>star fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean AUC</td>
<td>0.23±0.06</td>
<td>0.33±0.06*</td>
<td>0.39±0.11</td>
<td>0.37±0.12*</td>
</tr>
</tbody>
</table>

Conclusions: Pomegranate juice, star fruit juice and simvastatin influenced the pharmacokinetics of carbamazepine in rat. These treatment did not affect the 1.3-fold values of carbamazepine and metabolite formation in the systemic circulation. These results suggest that the interactions would be caused by enteric CYP3A inhibition.

Cost Implications of Oral Treatment of Colorectal Cancer in Germany

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Background: Fluoropyrimidine-based therapies are standard in the treatment of metastatic colorectal cancer. Intravenous 5-FU was the only option before Capecitabine, an oral fluoropyrimidine, became available. As the drug acquisition costs of Capecitabine are considerably higher than those of 5-FU, the question of using an oral agent became relevant. This led to the study evaluate cost implications of oral chemotherapy with Capecitabine vs. standard fluoropyrimidine-therapies (Mayo setting).This study compared 5-FU and Capecitabine in a German hospital setting.

Methods: From fluoropyrimidine-therapies were evaluated for the office-based setting. Physician’s fees (89 quarterly fee-listings, 26 patients, 6 office-based oncologists), drug and pharmacy costs and costs for venous port systems and single-use pumps were included. Capecitabine treatment costs were assessed to be identical to the cost of the equivalent oral fluoropyrimidine, except drug administration and acquisition. Based on the frequency of administration of active drugs by office-based oncologists costs were modelled for the hospital sector, i.e. day-case and inpatient treatment. A third setting was evaluated which was given in hospital. An additional cost data on frequency and setting of use of the evaluated regimens were used to estimate potential overall cost implications.

Results: Treatment costs for a 6-months course in the office-based setting was most expensive with the AI0/Aralant regimen (€18’600) and cheapest with Capcetabine (€3’975). Treatment costs in the hospital setting ranged from €7’070 (Mayo) to €22’790 (Aralant, inpatient treatment).

Conclusions: Higher drug acquisition costs of Capecitabine compared to 5-fluorouracil are more than compensated by lower costs for drug administration, resulting in net cost savings for Capcetabine. The most expensive treatment scenarios were the AI0/Aralant-control in both settings. Patient transfers to oral capcetabine is likely to result in substantial cost savings. Savings are likely to be even higher if combination therapies with irinotecan or oxaliplatin are considered.
A new focus on atherosclerosis treatment: transsialidase from Trypanosoma cruzi as an anti-proliferative drug.

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Background – Chagas disease is caused by Trypanosoma cruzi that produces transsialidases (TS) and Chagas patients usually do not have atherosclerosis. Atherosclerosis is an inflammatory multifactorial disease, presenting increased levels of sialic acids (SAs), transferin and iron in the plaques possibly related with free radical release and inflammation. TS produced by TC may remove SAs from the plaques.

Aim: To report a new way for treating atherosclerosis focusing on multigard targets simultaneously: removal SAs with a recombinant TC TS, a metal chelator (PDTC) and antioxidative nanoparticles derived from three plant extracts in an animal model.

Methods – We compared six groups (n=5) of rabbits. GI – normal diet; GII – 1% cholesterol diet for 12 weeks; GIII – 1% cholesterol diet for 12 weeks and, in the last 4 weeks, injection of transsialidase (TS) plus PDTC. Groups IV, V and VI received the same scheme of GIII plus aged extracts: Allium sativum (AL); AL+ Ginko biloba (GB) and AL+GB+ Zingiber officinale (ZO), respectively. The thoracic aorta atheromas stained with Sudan IV were macroscopically detected and plaque and fat areas in cross sections were microscopically detected using an image analysis system. The LDL in the serum was also measured.

Results – The combined therapy using TS+PDTC and 3 plant extracts was the most effective scheme in reducing atheromas and LDL serum levels to normal values. (Figure and Table below).


Consequences to morphology of primary neurons as an effect of Nitric Oxide on plasma membrane fluidity

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Nitric Oxide is described as a signal molecule of special importance in the nervous system mainly acting via cyclic guanosine monophosphate which is generated by the Nitric Oxide sensitive guanylate cyclase. However, our group [Grone Westrick, Hippe et al. (in preparation)] could show that Nitric Oxide has a direct effect on the plasma membrane of living cells by demonstrating the same effect on artificial proteoliposomes. In consequence of Nitric Oxide donor application membrane fluidity is increased.

Initial experiments have shown that the same effect occurs on neuronal cells. By the use of NOC-18 (Nitric Oxide donor) and L-NAME (Nitric Oxide synthase inhibitor) we show expected morphological changes on primary hippocampal neurons with scanning electron microscopy and immunocytochemical staining in addition to enhanced membrane fluidity measured by fluorescence recovery after photobleaching (FRAP). Further we plan to establish solid state 29P-NMR anisotropy measurements of artificial generated liposomes followed by electron microscopy analysis to detect changes in the membrane of those vesicles. In combination with mass spectrometry measurements we intend to demonstrate the molecular basis of the characterized direct NO effect on neuronal plasma membranes.

Changes in Plasma Protein Binding of an Extensively Bound and Highly Extracted Drug, Propofol, Have Clinical Relevance

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Background: Changes in plasma protein binding have little clinical relevance for most drugs. However, this situation is likely to be clinically important for a limited number of highly cleared drugs that are extensively protein bound, administered intravenously and have a narrow therapeutic index. Propofol has been widely used in the clinical field of anesthesia and intensive care medicine and is one such drug. Thus, changes in plasma protein binding of propofol would be expected to alter disposition of propofol and its potency. Therefore, we have investigated propofol’s pharmacokinetics during cardiopulmonary bypass (CPB) to determine whether the predicted theoretical changes actually occur.

Methods: After induction of anesthesia propofol was infused continuously during surgery. Propofol’s concentration was measured by HPLC in blood samples collected from the radial artery during surgery at predetermined time points. The drug’s unbound fraction in arterial plasma was estimated via equilibrium dialysis. Bispectral index (BIS) and burst suppression ratio (BSR) were measured continuously to quantify the potency of propofol.

Results: The total concentration of propofol in blood was unchanged during surgery. By contrast, the fraction of unbound propofol in blood increased by 2-fold during cardiopulmonary bypass. BIS was significantly decreased and BSR was significantly increased during CPB.

Conclusions: The potency of propofol significantly increased during CPB without any alteration in the total drug concentration. The enhanced efficacy would be caused by a reduction in plasma binding of the drug. Furthermore, total drug levels of propofol are influenced by cardiac output. We also report the cases of accidental hemorrhagic shock in patients undergoing liver transplantation, where 2-fold increases in both total propofol concentrations and the unbound fraction result in 4-fold increases in the unbound concentrations. Changes in plasma protein binding of propofol would be clinically important.
Rational Design of Specific Inhibitors of γ-Glutamyl Transpeptidase (GGT) and γ-Glutamylcysteine Synthetase (GCS) for Modulating Cellular Glutathione and Redox Status

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Background: Glutathione (γ-Glu-Cys-Gly, GSH) is a ubiquitous tripeptide that serves as a major cellular antioxidant and detoxifying agent. Therefore cellular GSH level is deeply associated with drug resistance of cancer cells and pathogens. GSH biosynthesis is dependent on the activities of γ-glutamylcysteine synthetase (GCS), a second biosynthetic enzyme, and of γ-glutamyl transpeptidase (GGT) that hydrolyzes the extracellular GSH to supply cells with its constituent amino acids. Aims: 1) To develop rationally specific inhibitors of GGT and GCS. 2) To understand the interaction of the inhibitors with the enzymes at the molecular level.

Methods: A series of electron-rich γ-phosphonic diester analogs of GSH were synthesized as mechanism-based inhibitors of GGT. Each inhibitor was evaluated by the second-order rate constant for enzyme inactivation. The structure-activity relationships were used for the active-site mapping. For GCS inhibitors, the recognition of Cys by an Arg residue of E. coli GCS was utilized for the inhibitor design, and a cyanogen group was introduced as a SH mimic.

Results: The γ-phosphonic diesters served as potent irreversible inhibitors of both E. coli and human GGTs by attaching covalently with the catalytic Thr. The potency was highly dependent on the structure mimicking the Cys-Gly moiety, but human GGT was far more selective, suggesting that human GGT served a "glutathionase" in vivo. The γ-phosphonic diesters did not inhibit glutamate amidotransferases. The most potent GGT inhibitor exhibited ca. 6000 times as potent as acivicin, a hitherto used non-selective GGT inhibitor. The introduction of CN group significantly increased the potency of inhibitor for both E. coli and a pathogenic Streptococcus GCS. The best inhibitor was ca. 2500 times more potent than BSO, a commonly used GCS inhibitor.

Conclusions: The reaction mechanisms and the structure of GSH biosynthetic and degrading enzymes were highly useful for rational design of selective and potent inhibitors. This is the first step toward controlling the cellular GSH levels that is highly promising for combating drug resistance.

Effective selectivity and compounds of treatment for childhood liver tumors – from the experience of JPLT (Japanese Study Group for Pediatric Liver Tumor) study

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Background: Hepatoblastoma (HB), which is derived from hepatic precursor cells, is a primary liver cancer that occur predominantly in children. The prognosis of children with HB has been improved significantly during the past two decades by the induction of neoadjuvant and adjuvant chemotherapy. JPLT group launched in 1991 is running cooperative treatment studies based on the sipatrigatinibulin regimen (CITA) as the first line on HB. Aims: 1) To evaluate the efficacy of JPLT protocol. 2) To evaluate the late complications of the patients by JPLT regimen.

Methods: Until 2007, 235 HB cases have been registered in JPLT-2. According to PRETEXT classification, standard risk (SR) tumors (PRETEXT 1-3) without metastasis were 146 and high risk (HR) patients (PRETEXT-4 or metastatic cases) were 89. In JPLT-2 protocol, high dose chemotherapy with stem cell transplantation (SCT) was carried out for metastatic tumors and living-donor liver transplantation (LD-LT) for some PRETEXT-4 tumors. Late complications were evaluated in 126 cases that received complete regimen and survived more than 2 years.

Results: The 3 year overall survival (OS) and event-free survival were 80% (95% in SR and 54% in HR) and 67%, respectively. The response rates of CITA regimen was 82% and 52% and the complete resectability was 87% and 41% in SR and HR cases, respectively. In 25 cases with metastatic disease, only 13 cases (57%) were cured. In 31 cases who underwent LD-LT, 3-year OS was 76%. Late complications are shown in Table.

Long term toxicity

<table>
<thead>
<tr>
<th>Grade 1-2</th>
<th>Grade 3-4</th>
<th>Grade 5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth &amp; development</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Cardiac</td>
<td>11</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Auditory</td>
<td>17</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Secondary malignancies</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Conclusions: 1) SR cases had a fair to excellent outcome but, late complications are now problematic. 2) The outcome of HR cases remained poor. Reduction of chemotherapy for SR cases and more promising strategies for HR cases including LT and new targeting drugs should be developed in international collaboration because the case numbers were limited.

System approach to Magic Bullets: tissue and cell targeted delivery of HIV and cancer drugs

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The estimated number of drug targets between 1940-1990 was about 500. In post-genomics era, over 1700 human genes express function proteins suitable for serving as drug targets. Despite the drug-target growth, approval of novel anticancer and anti-HIV therapeutics for human diseases has not increased substantially. In addition to well-documented preclinical challenges (in drug absorption, distribution, metabolism and elimination) in the development of new drug candidates, increasing frequency in drug failures during the late-stage clinical trials are often attributed to toxicity or lack-of-efficiency. Consequently, introduction of new drugs for the market is far below increased expenditure by pharmaceutical industry and government sponsors. To improve success rate, distribution profile of drug targets to "normal" and "cancerous or virus-infected" cells are being elucidated and mapped with the help of various in vivo-imaging techniques as a part of increased collaborations among basic, preclinical and clinical scientists, as well as introduction of translation research in early drug discovery and development. Even with improved understanding in "biodistribution of drug targets," oral or systemically administered drug molecules must cross a number of physiologic—tissue, cell and enzyme (as cytochrome P450's metabolism in the gut and liver) barriers before reaching the virus or cancerous cells found in target tissues and cells. A significant fraction of drugs are therefore eliminated in the gut and liver without ever reaching systemic blood circulation or being metabolized and inactivated. Some of the metabolites also induce unfavorable responses in liver kidney and other tissues. As a result, current oral anti-HIV drug combination therapies could reduce virus load to undetectable levels in the blood, but could not clear the virus in the tissues such as those in lymphoid tissues and cells. Building on the physiologic and biologic understanding at systemic levels, and applying the advances in drug delivery technologies, we have made progress in improving drug localization at three progressive levels; 1) lymphoid tissues, 2) HIV host cells, (3) HIV drug targets—protease and reverse transcriptase found in HIV infected cells.

This presentation will highlight our results in developing a systematic, practical, and novel approach to accomplish these goals using a HIV infected macaques model.

Authors' disclosure statement: Supported by NIH grants AI077390; AI52663; GM02883; NS39178, and Mito Gilabl Endowment Fund.
Pharmacokinetic/pharmacodynamic considerations for inhaled glucocorticoids

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Background: For the last 30 years, pulmonary drug delivery has been successfully employed for topical therapy of pulmonary diseases, with the goal of achieving pronounced pulmonary effect while reducing systemic side effects. The degree of pulmonary targeting is determined by a number of pharmacokinetic (PK) and pharmacodynamic (PD) factors. This presentation will discuss these relationships, and will review the pharmacokinetic and pharmacodynamic properties an inhaled glucocorticoid should provide.

Methods: This presentation is based on previously published in-vitro experiments, PK/PD simulations, animal and clinical studies of glucocorticoid action including the effects of biopharmaceutical parameters on pulmonary selectivity.

Results: Pharmacokinetic/dynamic simulations suggested that pharmacodynamic properties of an inhaled glucocorticoid beneficial for pulmonary targeting are low oral bioavailability, pronounced systemic clearance and distinct pulmonary residence time, while factors such as protein binding and degree of receptor affinity can be adjusted for by dose. PK/PD tools were also suitable to address the question when once-daily glucocorticoids should be administered. A pulmonary targeting model in rats was able to demonstrate the relevance of the results obtained in PK/PD based simulations and revealed the importance of biopharmaceutical optimization for the degree of pulmonary selectivity.

Conclusions: The presented work suggests that the use of PK/PD tools within the rational drug design is also beneficial when drugs for topical use, such as inhalation, are to be designed.

Authors' disclosure statement: Studies were supported in part by GSK and Astra-Zeneca.

The Utility of the Cholesterol:Cholesterol Ratio in Predicting LDL-Cholesterol response to Atorvastatin 80 mg: A paradigm for individualized lipid-lowering therapy

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Background: Cholesterol is an endogenously produced marker of cholesterol absorption that has been validated with sterol balance methodology. A retrospective analysis by the SPRINT investigators suggested the hypercholesterolemia phenotype in the highest quartile of the cholesterol:cholesterol ratio (CCR), indicating a cholesterol ‘absorber’ phenotype did not benefit from statin therapy in the 45 trial. Patients with high CCR have high cholesterol absorption and low synthesis; hence statins are expected to be relatively ineffective in such patients. Since recent meta-analyses have shown that benefit from statin therapy is directly related to LDL-cholesterol (LDL-C) response, we sought to prospectively assess whether the CCR can predict LDL-C response to Atorvastatin and identify statin responders, defined as an LDL-C response <40%.

Aims: To assess the predictive value of CCR in predicting LDL-C response to Atorvastatin 80mg.

Hypotheses: CCR correlates with the LDL-C response to Atorvastatin. Patients who have a higher CCR will have a suboptimal LDL-C response to statin and the CCR can be used to identify statin hyper-responders, defined as an LDL-C response <40%.

Methods: >40 patients with coronary artery disease or coronary risk-equivalents were recruited into the study. Baseline measures included CCR, baseline lipid panel, insulin, CRP, assessment of metabolic syndrome criteria, blood sampling for genetic analysis and an MRI substudy for patients without contraindications. Patients were given a 6 week course of Atorvastatin 8mg before follow up to determine the lipid and CRP response to the course of therapy.

Major Results: The 6 week course of Atorvastatin reduced LDL-C by a mean of 55.7% (P<0.01 for post vs pre-treatment LDL-C values), however, there was marked heterogeneity in the LDL-C response (range 12-80%). Of the baseline variables assessed, only the CCR correlated with the percent LDL-C reduction (r=0.20, P<0.01). When the population was divided into quartiles of CCR, statin hypo-responders (LDL-C response <45%) clustered in the patients with the highest CCR (quartile 4 vs quartile 1 p<0.03). Patients in the highest quartile (quartile 4) of CCR showed less features of the obesity and the metabolic syndrome than those in the lowest quartile (quartile 1) with significant differences being found in number of metabolic syndrome criteria, HDL-C, LDL-C, TG, BMI (all p<0.01). ROC analysis suggested that the CCR can identify statin hypo-responders (LDL-C response <40%) with a 100% sensitivity and 62% specificity (Area under curve = 0.85, p<0.01).

Significance: This is a highly significant finding and highly applicable to personalized medicine in the area of lipid-lowering therapy. Of note, pharmacogenomic approaches to variability in statin response typically yield R2s of 0.01-0.02. We propose that the CCR can be used to predict LDL-C response to statin therapy and identify statin hypo-responders who might be expeditiously treated with anti-resorptive co-therapy such as ezetimibe. These findings are particularly applicable to high risk cardiovascular patients where aggressive lipid-lowering therapy is warranted.

Abstracts
Heterocyclic hydrazones induce radical formation and dissipation of mitochondrial membrane potential

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Background: The novel compound N-benzoazo-2-yl-N'-1-(isoquinolin-3-yl-ethyldiene)-hydrazine (EPH136) has been shown to exhibit antitumor activity in vitro and in vivo. A COMPARE analysis showed that the patterns of cellular effects of EPH136 are not related to any of 175 standard antitumor agents with a known mechanism of action.

Methods: In order to help identify the mechanism of action we employed the following methods: (i) a bioinformatics approach, called partial least squares modelling in latent variables, (ii) a DNA microarray for detection of the expression of ~5000 known genes following treatment of HT-29 colon carcinoma cells with a two-fold IC50 concentration of EPH136, (iii) detection of radicals by luminol, (iv) determination of the mitochondrial membrane potential by JC-1 and by TMRE, (v) treatment of cells with EPH136 and the radical scavenger N-acetylcytochrome and (vi) treatment of cells with elevated levels of glutathione.

Results: The 60 genes found by the bioinformatic approach to be most important for the antiproliferative effect of EPH136 are involved in nucleoside, nucleotide, nucleic acid binding and metabolism, developmental processes, protein modification and metabolism. The genes that were up-regulated more than two-fold (in DNA microarrays) compared to untreated controls belong to the same classes as found by the bioinformatic approach. Many of these proteins are regulated by oxidation/reduction and so we concluded that formation of radicals may be involved in the mechanism of action. We found that EPH136 leads to generation of radicals, swelling of mitochondria and dissipation of the mitochondrial membrane potential. The antiproliferative effect of EPH136 was prevented by the radical scavenger N-acetylcytochrome. Cells with elevated glutathione exhibited resistance to EPH136.

Conclusions: The mechanism of action of the novel experimental anticancer drug EPH136 is generation of radicals and dissipation of the mitochondrial membrane potential.
Inhibitors of Serine/Threonine Protein Phosphatases at the Dawn of a Clinical Era.

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The reversible phosphorylation of proteins regulates the biological activity of many diverse cellular processes, including gene transcription, protein-protein interactions, cell-cycle progression and apoptosis. Phosphorylation occurs principally on serine and tyrosine residues, with the addition and removal of phosphate catalyzed by kinases and phosphatases, respectively. Although protein phosphatases were once viewed as simple housekeeping proteins, in the last decade it has become eminently clear that they are actually dynamic and highly regulated enzymes. Therefore, the development of compounds that alter the activity of key phosphatases is rapidly emerging as an important area in drug discovery. Because the majority of protein phosphorylation (>90%) occurs on serine and threonine residues, the identification of specific agents that alter the activity of key ser/thr phosphatases seems especially promising for development. For the PPP-family of phosphatases, several lead compounds for drug development have come from studying the biological actions of natural products, such as fostriecon and cantharidin, which was identified as an activate constituent catalyzed by kinase and phosphatase, respectively. Although protein phosphatase were once viewed as simple housekeeping proteins, in the last decade it has become eminently clear that they are actually dynamic and highly regulated enzymes. Therefore, the development of compounds that alter the activity of key phosphatases is rapidly emerging as an important area in drug discovery. Because the majority of protein phosphorylation (>90%) occurs on serine and threonine residues, the identification of specific agents that alter the activity of key ser/thr phosphatases seems especially promising for development.

What Sort of Light Is At the End Of the Tunnel In Anti-Allergic Drugs?

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Background: Studies on the efficacy of anti-allergic drugs in the treatment of allergic rhino-conjunctivitis have yielded inconsistent and unsatisfying results, notwithstanding their approval for this indication.

Methods: In consecutive single-centre, double-blind, placebo controlled, cross over studies, more than 500 subjects with confirmed allergic rhinitis due to grass pollen or house dust mite were randomised to receive active or placebo drugs and exposed to the account allergen (grass or mite) in the Vienna Challenge Chamber (VCC) for several hours. Patients recorded symptoms at 15-minute intervals, nasal secretion and nasal resistance were evaluated every 30-minutes. The primary endpoint was the mean change from placebo in total symptom score.

Results: All of the investigated compounds like H1-receptor antagonists, topically used steroids and other mediator antagonists were significantly different from placebo in the primary endpoint but not very different in their therapeutic power. However, all of the approved drugs did not reach a therapeutic potency of more than 35% over placebo reflecting the frustration of many patients suffering from allergic rhinitis. Never the less, some compounds, out of the pipeline, have overcome this threshold, whereas others reaching the target were discontinued in their development, due to the risk/benefit viewpoint.

Conclusion: The standardised and validates model of an allergen challenge chamber like the VCC enables us to compare results from different trials on a historical basis. Albeit approved anti-allergic drugs do not fully satisfy suffering individuals, several new compounds are under investigation and hopefully reaching a risk/benefit ration which will be acceptable for the patients.
Antitumor Effect of a Novel NF-κB Targeting Therapy in Bladder Cancers

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Background: Nuclear factor (NF)-κB is a transcription factor not only induces and controls various genes including inflammatory cytokines, but also activates genes with antipapoptotic function, proliferation and invasion. It has been demonstrated that certain advanced human bladder cancer cells constitutively acquire the ability to activate NF-κB, which not only protects cancer cells from apoptotic cell death, but also up-regulates the production of various cytokines that might induce malignant potential of the disease. NF-κB activation inhibitor, therefore, might be useful as novel anticancer agents. We have designed and synthesized a NF-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ) and investigated the effectiveness of DHMEQ against advanced human bladder cancer cells, KU-19-19 in which NF-κB is constitutively activated.

Methods: KU19-19 was implanted s.c. in the flank of nude mice. Daily i.p. administration of 2 mg/kg DHMEQ was started from 7 days after tumor implantation. After 28 days, mice were sacrificed and all the tumors were evaluated. Microvessels in tumor specimens were counted after immunostaining with an anti-CD34 monoclonal antibody. Apoptosis was measured by TUNEL assay using Apoptosis in situ Detection Kit. The average number of positively stained cells was counted and apoptosis index was calculated.

Results: inhibition of NF-κB by transfection of adenovirus vectors expressing stable form of NF-κB inhibitor, IκBα, inhibited KU19-19 cell growth and induced apoptosis. DNA binding activity of NF-κB was completely inhibited by DHMEQ. Marked levels of apoptosis were observed after DHMEQ administration. DHMEQ treatment in vivo inhibited KU19-19 tumor growth. Tumor volume after DHMEQ treatment was 2110±491 mm³ vs 6019±2309 mm³ of control mice (P<0.05). A statistically significant decrease in MVD in DHMEQ-treated tumors was observed. Blood vessels in the tumors derived from control mice showed well-developed vascular networks. In contrast, the vessels in the tumors of DHMEQ-treated mice consisted of poorly developed networks. The apoptotic index was increased 2.3-fold in DHMEQ-treated tumors in comparison with control tumors.

Conclusions: Targeting NF-κB could be a new strategy of treatment against advanced bladder cancer.

The Clinical Pipeline of a Candidate Malaria Vaccine that Targets the Achilles’ Heel Antigen of Plasmodium falciparum

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Background: Malaria remains as one of the most infectious disease globally. Failure of existing control strategies necessitates the need for vaccine development. We focused on the development of an effective vaccine using a recombinant SE36 protein based on the N-terminal domain of Serine Repeat Antigen (SERA5) of Plasmodium falciparum.

Methods: In vitro studies have elucidated the transcription profile, processing and localization of SERA5. A recombinant version of the 47 KD N-terminal domain, and a modified version with high hydrophilicity (SE36), has been produced in E. coli. Molecular and immunology based epidemiological studies have been conducted in Uganda and Solomon Islands. An efficient fermentation and purification process has also been developed for the production of this antigen in a scale comparable with industrial manufacture and this has been used for preclinical and toxicology studies in animals to demonstrate safety and immunogenicity. The candidate vaccine, BK-SE36, was formulated adsorbed to aluminium hydroxide gel and is manufactured as a lyophilized product under Good Manufacturing Practices (GMP). A phase 1a clinical trial has been conducted in Japan.

Results: In vitro, several lines of evidence suggest that SERA5, which belongs to a multigene family unique to Plasmodium, plays an essential role in parasite development and merozoite egress. Immunoproteomics data underscores the uniqueness of SERA: naturally induced antibody response to SE36 protein correlated with increased protective immunity in adults and children. Higher levels of anti-SE36 IgG3 titer were associated with the absence of fever and lower parasitemia in children under 15 years; and were associated with protection against severe malaria in children under 5. Sero-conversion rates were 50% or less in >16 yr-old; and less than 10% in <10 yr-old individuals. Preclinical studies in animals showed that BK- SE36 was safe and highly immunogenic. Immunological test using squirrel monkeys provided significant protection after Pl. falciparum challenge infection; and antibody titers were significantly boosted. BK-SE36 was, likewise, safe and highly immunogenic in chimpanzees. No significant safety issues have been identified in healthy, malaria-unexposed adults in a Phase 1a clinical trial in Japan. Cumulative data confirms the potential of this candidate vaccine.

Characterization of an Active Pharmaceutical Ingredient by Its Dissolution Properties: Amoxicillin Trihydrate as a Model Drug

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Background: An ordinary powder sample of an active pharmaceutical ingredient (API) is composed of particles having various properties with respect to their shapes, dimensions, etc. When taking into account such differences in particles, the dissolution of a sample can be described using a diffusion theory of heterogeneous particle populations, where every population is characterized by a particle size coefficient (α), consisting of a geometric factor and a material constant. Aims: 1) To develop a requested theory as a system of differential equations (SDE’s). 2) To prove the applicability of the theory for sieved fractions of API. 3) To predict dissolution profiles for samples imported from foreign markets were compared with Japanese DTPa’s for injection site injuring effects in mouse footpad swelling, rabbit skin swelling and mouse quadriceps muscle injection models. Regarding local reaction to a booster dose, mice were intramuscularly immunized with a vaccine sample twice at one month interval and challenged with 50 microgram of diphtheria toxoid to measure swelling reaction.

Results: Annually two mortality and three encephalopathy cases, in average, were reported among over five million doses of diphtheria toxoid whole cell pertussis vaccine (DTPa). However, reports of such severe AEi have become quite rare after 1991 when minimum requirements of biological products (MRBP) for DTPa was revised to strengthen detoxification of pertussis toxin (Table 1) to suggest relevance of DTaP with the rare but severe AEFI.

Regarding local reaction, all imported vaccines induced very strong inflammation and tissue injury at injection sites of mouse footpad, rabbit skin and mouse quadriceps muscle while Japanese DTPa induced no such reaction to suggest differences in tissue damaging effect of the vaccines. We evaluated enhancing effect of DTaP with varied levels of residual PT activity on local reaction to a booster dose using mouse footpad swelling model. A significant correlation was seen between mouse footpad swelling and residual PT activity of immunized vaccine batches.

Conclusions: 1) Reduced toxicity in laboratory tests seemed to be relevant to safety, 2) Clinical observation could not evaluate injection site injury. 3) DTaP may enhance the local reaction to booster doses. 4) Not only clinical evaluations, laboratory models need to be focused on in vaccine evaluation.
EHLRI II – 2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich
Nürnberg, October 3-5, 2008

Estradiol as Membrane Targeting Modulator of Neuronal Cell Functions

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Background: Accumulating evidence from basic science studies demonstrates that estrogens exert profound protective actions against various forms of neurodegenerative diseases and injury. Recently, evidence has accumulated in favour of membrane effects of estrogens due to the increased membrane protein content, which may specifically bind to membrane targets. One example of estrogen modulation of membrane properties involves the modulation of intracellular calcium homeostasis. In our earlier as well as recent unpublished study both modulation of intracellular calcium stores and regulation of plasma membrane calcium channels have been proposed. We examined in vitro effects of 17β-estradiol (E2) on intracellular calcium ([Ca2+]i) concentration in isolated presynaptic nerve terminals (synaptosomes) from whole female rat brain and discrete brain regions by measuring time-dependent effects of E2 on Ca2+ influx in synaptosomes through voltage-gated channels and extrusion of Ca2+ by sodium/calcium (Na/Ca) exchanger.

Methods: Synaptosomes were isolated from whole brain (WB), brain stem (BS), nucleus caudatus (NC) and hippocampus (Hip) of chronically overdosed three-month-old female rats (18 animals/study group/preparation). Binding of E2 to isolated synaptosomal plasma membranes (SPM) and synaptosomal mitochondria were calculated by subtracting non-specific (in the presence of 100-fold excess unlabelled E2) from total binding of [3H]-estradiol (E2) (10-10 - 10-6 M). For Ca2+ influx measurement synaptosomes were preincubated in the presence or absence of E2 (10-10 - 10-6 M) for 15 min. The voltage-dependent "Ca2+" influx were measured in the presence of 50 mM KCl and the Na-dependent Ca efflux in resting (4 mM KCl) for 30 sec. Relaxed "Ca2+" in synaptosomes were determined by radioactivity measurement after filtering synaptosomal pellets through nitrocellulose filters (0.45 µm pore size). Synaptosomal mitochondria were preincubated with or without E2 (10-10 - 10-4 M) for 10 min and "Ca2+" efflux through uniporal as well Na-dependent Ca efflux (in the "Ca2+" preloaded mitochondria in presence of 20 mM NaCl and 0.2 mM EDTA) were measured.

Results: E2 bound to two specific sites on SPM from NC (Bmax 161.5±1.9 pmol/mg, Kd 21.5 nM) and NC (Bmax 13.8±1.3 pmol/mg, Kd 25.2 nM). For Ca2+ influx measurement synaptosomes were preincubated in the presence or absence of E2 (10-10 - 10-6 M) for 15 min. The voltage-dependent "Ca2+" influx were measured in the presence of 50 mM KCl and the Na-dependent Ca efflux in resting (4 mM KCl) for 30 sec. Relaxed "Ca2+" in synaptosomes were determined by radioactivity measurement after filtering synaptosomal pellets through nitrocellulose filters (0.45 µm pore size). Synaptosomal mitochondria were preincubated with or without E2 (10-10 - 10-4 M) for 10 min and "Ca2+" efflux through uniporal as well Na-dependent Ca efflux (in the "Ca2+" preloaded mitochondria in presence of 20 mM NaCl and 0.2 mM EDTA) were measured.

Conclusions: E2 at physiological concentrations specifically bound to SPM and synaptosomal mitochondria from discrete brain regions and at same concentrations mimicked voltage-dependent influx and Na-dependent efflux of calcium in synaptosomes and decreasing mitochondrial Ca release.

Dioxin like compounds for anti-infective agents of know as its degrading enzyme from its resistant Geobacillus midousuji thermophile

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Background: Dioxins are mostly created by human activity and can be classified: i. Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs); ii. Polychlorinated biphenyls (PCBs); iii. Polycyclic aromatic hydrocarbons (PAHs). We have noticed Dioxins are kinds of anti-inflammatory and anti-mitotic agent and anti-proliferative agents through its resistant bacteria with its respiratory activity. Organic cation transporters (OCTs) have an important role in tissue distribution and elimination of dioxin-like drugs. Carrier-mediated disposal of dioxin-like compounds in the liver is, however, incompletely understood. Aim: To assess the uptake of long-acting [14C]-agonists by bronchial and vascular smooth muscle cells (SMCs).

Methods: Human airway cells and tissues obtained from organ donors were evaluated for cationic drug transporter expression by quantitative RT-PCR and immunofluorescence. In vitro functional studies, (3H)-formetorol (FORM) and (3H)-salmeterol (SALM) uptake by bronchial and vascular SMCs was measured.

Results: RT-PCR analysis indicated high mRNA levels for the corticosteroid-sensitive OCT3 in bronhial and vascular SMC. Immunofluorescence staining of airway sections confirmed OCT3 expression in these cells. In bronchial SMC, uptake of the cationic FORM was inhibited with OCT3 inhibitors. Corticosteroids also inhibited FORM uptake through a rapid (within 15 min) nongenomic action, with the following rank order (relative potency): des-ciclosporine (11.1) > ketoconazole (5.2) > beudesonide (3.8) > beclometasone dipropionate (1.7) > ciclesonide (1.4) > fluticasone (1). The corticosteroid-induced inhibition was significantly higher in vascular than bronchial SMCs. In comparison to FORM, uptake of the noncharged lipophilic SALM was about 10-fold higher (28.4 ± 1.7 vs. 327.5 ± 13.7 pmol/mg/15 min; p ≤ 0.05), and insensitive to OCT inhibitors and corticosteroids.

Conclusions: Our findings suggest that corticosteroids, through OCT3 inhibition, rapidly interfere with drug disposal mechanisms in the airway. Increased tissue retention of inhaled cationic bronchodilators due to the corticosteroid-sensitive disposal mechanism could acutely improve bronchodilator responses. This novel interaction supports the use of such combinations in asthma therapy.

The prevalence and the resistance mechanism of florfenicolines in bacteria isolated from Bangladesh

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Background: Antimicrobials have been a much misused product in the world. In Bangladesh only 8% of the total used antibiotics are prescribed by a physician; and in poultry, veterinary and aquaculture are using unknown quantities of large amount antibiotics in an uncontrolled manner. This gives rise to the emergence of antibiotic-resistant bacteria. Our group has been working on understanding the resistance of antibiotic-resistant bacteria. We present our work on understanding the resistance of antibiotic-resistant bacteria. We have been working on understanding the resistance mechanism of antibiotics, resistant bacteria against antibiotics and our mechanism of actions.

Methods: To address the problems, we studied following parameters in hospitals wastewater (HWW):i. correlation of antibiotics used by the patients and resistant bacteria development; ii. effect of wastewater on sensitive bacteria; and iii. functional studies, [3H]-formoterol (FORM) and [3H]-salmeterol (SALM) uptake by bronchial and vascular SMCs was measured.

Results: The bioassay using sensitive Escherichia coli 2 clearly revealed that HWW contained active antibiotics at higher concentration than MIC for sensitive bacteria. Enumeration of total resistant bacterial count showed that the count was about 10-log higher in HWW. We randomly selected 52 E. coli isolates with high multi-drug resistance including florfenicol (MIC > 600 µg/mL). DNA sequencing data revealed that ciprofloxacin resistance in bacteria occurs due to acquisition of mutations in gyrA gene. Computer modeling of the mutant and wild-DNA gyrase based on available DNA gyrase A crystal structure suggests that acquisition of double mutation that leads to alteration of the ciprofloxacin binding pocket may be the reason of high resistance properties shown by the isolates.

Conclusions: In our conclusion, we clearly demonstrate that i. HWW have ecotoxicological effect in spreading resistant bacteria as well as active antibiotics in the environments; and ii. florfenicolines resistance in bacteria isolated from Bangladesh are due to acquisition of mutation in gyrA gene rather plasmid born.

Abstracts

All abstracts are listed in alphabetical order of the presenting author.
Anti-neoplastic properties of tea catechins are associated with pro-differentiation caspase 14 gene expression: implementation for novel therapies


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Background: Exploration of novel approaches with innovative therapies is needed to combat epithelial cancer. We previously reported that caspase-14, a gene expressed during terminal differentiation of certain epithelial cells, is induced by green tea polyphenols. Our hypothesis is that the expression of caspase 14 induces tumor cell death without damaging normal epithelial cells. Therefore, caspase 14 is a suitable candidate for novel gene therapy to treat a variety of cancers.

Objectives: to express exogenous human caspase 14 in human epithelial cancer cells (OSCC) by plasmid transfection and adeno-virus delivery, and determine the effects of caspase-14 expression on cell growth, cell death, and tumorigenicity.

Methods: The human cancer cell lines A431, HSG, and OSCC were either transfected with caspase 14 expressing plasmid, or infected by adenovirus expressing caspase 14 cDNA. Expression of caspase 14 was confirmed by Western blotting. Cell morphology was monitored by microscopic photography, cell growth was measured by cell counting and BrdU assay, and cell viability was determined by MTT assay. In addition, the cancer cells were xenografted into athymic mice to determine the tumorigenicity.

Results: expression of caspase-14 induced an undefined cell death in these cancer cells compared to the control cells. Cell growth and cell viability were inhibited significantly by caspase-14 expression. Xenograft of caspase-14 expressing cancer cells into athymic mice resulted in significantly reduced tumorigency, which could due to an inhibitory effect of caspase 14 on tumor vascularization.

Conclusions: human epithelial cancer cells undergo growth inhibition and cell death when exogenous caspase-14 was expressed in these undifferentiated tumor cells. Caspase-14 expression in these cells also reduced tumorigenicity in vivo. Further effort is warranted to explore if caspase 14-expressing adeno-virus could be used as a potential therapeutic approach to treat human cancers.

This study was supported in part by the Dental Research Foundation of the Medical College of Georgia.

Development of species-specific STAMPs (specific target antimicrobial peptide) that target and kill only Streptococcus pneumoniae within a polymicrobial community

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Abstracts
Inhibitors in a Mice Metastatic Brain Tumor Model

Background: While adequate delivery of drugs may occur in systemic tumors, the blood-brain barrier (BBB) limits delivery of anti-tumor agents into brain tumors including metastases. In this study, we examined the role of phosphodiesterase type 5 (PDE5) inhibitors in BBB opening in a mouse metastatic brain tumor model.

Methods: Firstly, we established the metastatic brain tumor model by implanting CRL-5904 cells (human brain metastasis of non-small cell lung cancer) intracranially and flankly, but not in contralateral normal brain. In vitro drug uptake assay demonstrated that vardenafil enhanced the uptake of doxorubicin in both CRL-5904 and human brain microvessel endothelial cells (HBMEC). Silencing of the caveolin-1, an inhibitor of caveolae transcytosis pathway, could block PDE5 inhibitor-induced drug uptake, suggesting a mechanism via caveolae transcytosis pathway.

Conclusions: These findings suggest that PDE5 in metastatic brain tumors may serve as an effective target for pharmacological modulation of BBB permeability to enhance selective delivery of chemotherapeutic drugs to metastatic brain tumors.

Abstracts

The Impact of Inflammatory Responses on Taste Bud Cell Turnover and Function.

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Background: Taste disorders impact negatively on general health and quality of life. Although many conditions can contribute to or exacerbate taste deficits, viral and bacterial infections are among the most common causes. The underlying molecular and cellular mechanisms, however, are largely unknown. We hypothesize that inflammatory agents, particularly interferons (IFNs), may be involved in taste bud turnover.

Methods: Quantitative real-time polymerase chain reactions (qPCR) in situ hybridization and immunohistochemistry were used to determine the presence of IFN signaling pathways in taste buds. Primary tissue cultures of taste bud-containing lingual epithelia were stimulated with recombinant IFNs to assay the activation of these IFN pathways. Animal models mimicking the viral and bacterial infections were established to assess the impact of infection on gene expression in taste buds. Finally, the effect of systemic administration of IFNs on taste bud cell turnover was also evaluated.

Results: IFN signaling pathways, including IFN receptors and their downstream components: protein kinases JAK1 and TYK2, and transcription factors STAT1, STAT2, and IRF9 were present in subsets of taste bud cells. Incubation of recombinant IFNs with cultured taste tissues activated the IFN signaling pathways, leading to the phosphorylation of the transcription factors. Intraperitoneal injection of lipopolysaccharide or polyinosinic:polycytidylic acid up-regulated the expression of IFN-inducible genes in taste papillae whereas the systemic administration of recombinant IFNs resulted in the increased apoptosis in the taste buds.

Conclusions: These findings suggest that bacterial and viral infection-induced IFNs can act directly on taste bud cells, affecting their cellular function in taste transduction, and that IFN-induced apoptosis in taste buds may cause abnormal cell turnover and skew the representation of different taste bud cell types, which eventually lead to the development of taste disorders.

Combinational therapies Targeting Laboratory and Clinical isolates of HIV-1

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Background: Most if not all viruses, including replication-dependent mutants, are host dependent. They require the participation of certain cellular factors to sustain viral growth. Cellular factors, unlike viral proteins, are not under mutational pressure and are generally structurally invariant. Some of the viral motifs interacting with these cellular factors also remain evolutionarily stable. Thus, inhibitors that block the usage of these conserved factors at different stages of viral life cycle are likely to be good candidates for mutation-insensitive antiviral drugs.

Methods: A new cocktail of small organic molecules, targeted specifically at the highly conserved motifs of viral/host interactions at different steps of the viral life cycle (viral entry, integration, and pro-viral transcriptions) was developed. Using specific bioassay-guided purification methods, including counter current chromatography, Waters Co’s alliance HPLC 2690 separations module equipped with photodiode array detector, and empower chromatography manager, several classes of therapeutically important molecules have been isolated from plants and chemically identified.

Results: Structures of three anti-HIV inhibitors, GEN-1, M62, and GU targeting viral entry, integration, and pro-viral transcription respectively, are shown in Figure 1. When tested alone, GEN-1 has an IC50 of 40 μM, M62 an IC50 of 4.6 μM, and GU an IC50 of 18 μM. When tested in combination, they block HIV-1 production in culture C8-D cells synergistically against a variety of HIV strains at an IC50 of 1.3 μM. They are equally active in targeting mutant strains that are highly resistant to currently available drugs against HIV protease and reverse transcriptase as compared to the wild type viruses. In comparison, cytotoxicity for the three drugs combined in H9 cells was also analyzed using MTT assay. As further shown in Figure 2, cellular toxicity to H9 cells with combined was not detected in the presence of the three drugs, when 12 µM of each was used.
Drug-Peptide Conjugates with Antitumour or Anti-Parasite Activity

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Background: Peptide/protein carriers can be used as soluble drug conjugates for ‘passive targeting’ resulting in altered pharmacokinetics (e.g. accumulation in tumour), increased intracellular uptake, decreased non-specific toxicity and immunogenicity.

Methods: We have developed a new group of water-soluble oligo- or polypeptide-drug-conjugates in which daunomycin (Dau), methotrexate (MTX) or other antitumour or antiparasite agents are coupled to amphiphilic or polycationic branched polypeptides or to oligoarginine. Toxicity, in vitro and in vivo antitumour activity of conjugates with daunomycin were investigated using sensitive and multidrug resistant mouse (L1210 and P388 leukemia, S180 sarcoma, MXT breast carcinoma) and human (HL60 leukemia) tumors. The antiparasitic activity of the MTX containing conjugates was analysed also in vitro and in vivo using Leishmania donovani infected macrophages and animals.

Results: We found that attachment of the bioactive cargo significantly improved the antitumour or antiparasitic properties of the drug, respectively and also significant reduction of drug related side effects could be demonstrated.

Conclusions: The covalent-conjugation of drugs with amphiphilic or polycationic coating to oligoarginine or macromolecular polypeptide typer carrier could be useful strategy to develop new compounds with improved therapeutic efficacy.

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Background: The central nervous system and peripheral nerves are guarded against free access from the outside by the blood-brain, blood-cerebrospinal fluid and blood-nervous barriers. The glucose transporter GLUT1 mediates the specific transfer of glucose across these barriers while GLUT3 is a high-affinity isoform of Type I glucose transporter expressed mostly in neurons where it is believed to be the main glucose transporter isoform. As for a long time it was an open question whether GLUT1 and GLUT3 are present in the olfactory system the aim of the present study was to give answers to these questions.

Methods: In the studies including 20 male Wistar rats (4 weeks old) mucous membranes of the olfactory region were studied by double immunofluorescence labeling. Rabbit anti-GLUT1, guinea pig anti-GLUT1, rabbit anti-chicken tubulin, rabbit anti-GLUT3 and mouse anti-PGP served as primary antibodies. Fluorescein isothiocyanate-labeled donkey antirabbit IgG (IgG), dichlorotriazinyl amino fluorescein-labeled and rhodamine red X-labeled donkey anti-rabbit IgG, and Cy3-labeled donkey anti-mouse IgG were used as secondary antibodies.

Results: The studies indicated the abundant presence of GLUT1 in the endothelial cells of olfactory mucosa while the upper cells of olfactory epithelium (cellule neurosensóriales olfactórias) stained strongly positive for GLUT3. Anti-tubulin antibody strongly stained the apices of the olfactory epithelial cells as well as nerve fibers bundle emanating from the epithelium. Anti-PGP antibody stained olfactory receptor neurons in the olfactory epithelium and the nerve fibers running underneath.

Conclusions: The immunolocalization of GLUT1 in the endothelial cells of olfactory mucosa and GLUT3 expressed primarily in olfactory receptor neurons allows glucose to cross the blood-brain barrier and enter neurons. Moreover, the results also showed that PGP serves as a marker for the olfactory epithelium (nerve fibers emanating thereof) and that tubulin acts as a marker for the nerve fibers in olfactory mucosa.
Genetic Variant of KIF6 Predicts both Increased Risk for Coronary Events and Greater Benefit from Statin Therapy: An Overview of Genetic Studies of the CARE, WOSCOPS, and PROVE IT - TIMI 22 Trials

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Background: Statins are the drugs of choice for primary and secondary prevention of coronary heart disease; however, response to statin therapy varies dramatically between individuals. Aim: To identify and validate genetic polymorphisms that are associated with risk of coronary events and differential responses to statin therapy.

Methods: We used Cox proportional hazards models that adjusted for traditional risk factors to investigate the effect of pravastatin therapy versus placebo by KIF6 719Arg carrier status and association between KIF6 719Arg carrier status and coronary events in the CARE and WOSCOPS trials and to investigate the effect of high-dose atorvastatin therapy versus standard-dose pravastatin therapy in the PROVE IT-TIMI 22 trial.

Results: The 719Arg variant of Trp719Arg (rs20455), a polymorphism in kinesin-like protein 6, was associated with greater risk of coronary events and greater benefit from pravastatin versus placebo. In placebo-treated patients, carriers of the KIF6 719Arg allele (92% of CARE and WOSCOPS) had a hazard ratio of 1.50 (95% CI 1.24 - 1.80) as compared to non-carriers. Among 719Arg carriers, the absolute risk reduction by pravastatin was 4.1% (95% CI 1.8 - 7.5%) in CARE and 5.4% (95% CI 3.5 - 7.4%) in WOSCOPS. In contrast, no significant risk reduction was observed among non-carriers. In PROVE IT-TIMI22, benefit from high-dose, compared with standard-dose, statin therapy was significantly greater in the 59% of the cohort who were carriers (hazard ratio 0.56, 95% CI 0.45 - 0.77) than in non-carriers (hazard ratio 0.94, 95% CI 0.70 - 1.27; p<0.018 for interaction between 719Arg carrier status and treatment). Absolute risk reduction was 10.0% in carriers versus 0.8% in non-carriers.

Conclusions: 1) Carriers of the KIF6 719Arg allele are at increased risk of coronary events, and pravastatin therapy substantially reduces that risk. 2) Carriers of 719Arg receive significantly greater benefit from high-dose statin therapy than do non-carriers. 3) In all three trials, non-carriers of 719Arg (representing over 40% of the populations) did not benefit from statin therapy. Since the current benefit of other cardiovascular drugs, demonstrating the benefit of new cardiovascular compounds over highly potent statins may be more successful in statin non-responders identified by the KIF6 polymorphism.

Peroral Colon-Specific Delivery of Insulin Based on Novel Acrylic-Terpolymer Microcapsule Devices

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Background: Recently, colon has attracted much attention as a potentially delivery site for perorally administered peptide-based drugs. In this context, we have developed novel delayed-release microcapsules (MCs) with a membrane of newly synthesized acrylic terpolymers as a prototype of colon-specific delivery device for peptide-based drugs. Aims: 1) To prepare insulin-containing MCs. 2) To evaluate stability, in vitro release and in vivo absorption behavior of the microencapsulated insulin.

Methods: An aqueous colloidal dispersion of terpoly(ethyl acrylate/methyl methacrylate/maleic acid) was synthesized by emulsion polymerization. The MCs composed of a lactose core (95-105 µm), a layer of bovine pancreatic insulin with a protease inhibitor (bacitracin) and an absorption enhancer (sodium glycocholate), and a release-delaying coat of the terpolymers were prepared by the air-suspension spray coating process. The obtained MCs were heat-cured at 40 °C for 6 h and then subjected to stability assay by an HPLC method, release test using a paddle method and absorption study of insulin after peroral administration to gastrointestinal beagle dogs.

Results: The MCs with mass median diameters of 175–226 microns were obtained at the yield of 85–93%. Degradation of insulin during the spray-coating process and post-thermal curing was only few % when the process temperatures were set to below 40 °C. The heat-cured MCs showed delayed-release of insulin in a pH-independent manner. The lag-time of drug-release could be controlled by altering the coat thickness of terpolymers. Peroral administration of the MCs with 6 h-lag-time to the beagle dogs revealed a significant reducing effect of blood glucose level (the pharmacological availability was estimated to be 5.1%) while that of the MCs with 3-h lag-time did not. Moreover, the microencapsulated insulin was found to be stable and its release profile was not changed significantly even after the storage at 4°C for 2 years.

Conclusions: 1) Microencapsulation of insulin with the terpolymers was possible without significant degradation of insulin. 2) The microencapsulated insulin was stable for 2 years, released pH-independently in a delayed manner, and effective to enhance in vivo insulin absorption through the colon.

Oral Cancer: Molecular Pathogenesis and Novel Therapeutic Approach

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Background: Aberration of signal transducers in PI3K/AKT pathway has been found in many human cancers including oral cancer and may play a critical role in the etiology and progression of cancer. Advanced research on the treatments of oral cancer using novel agents targeting on PI3K/AKT signaling pathway are investigated in many laboratories with promising results. The objectives of the present study were (1) to investigate protein expression of pan AKT and its phosphorylated form, p-AKT, in oral squamous cell carcinoma (OSCC) tissues of 20 Thai patients, (2) to analyze mRNA expression of three isoforms of AKT, AKT-1, -2, and -3 and protein expression of pan AKT, AKT-1, and AKT-2 in OSCC cell lines and human oral keratinocytes (HOK), and (3) to analyze protein expression of vimentin and E-cadherin in OSCC and HOK cell lines.

Methods: The expression of pan AKT and p-AKT in OSCC tissues was studied by immunohistochemistry. The mRNA expression of AKT-1, -2, and -3 in OSCC cell lines and HOK was analyzed by RT-PCR and the protein expression of pan AKT, AKT-1, AKT-2, vimentin, and E-cadherin was studied by Western blot assay.

Results: The results showed that pan AKT and p-AKT were overexpressed in 95% and 100% of OSCC cases, respectively. We observed more intense expression of pan AKT and p-AKT at the invasive fronts of some OSCC tissues. Pan AKT protein was also overexpressed in all OSCC cell lines in comparison with HOK. Interestingly, AKT-1 and -2 mRNA of OSCC cell lines were only constitutively expressed in comparison with HOK. AKT-3 mRNA appeared to be minimally expressed in OSCC cell lines and HOK. The Western blot analysis revealed that AKT-2 but not AKT-1 was overexpressed. Addition of Activin A resulted in upregulated while E-cadherin was downregulated.

Conclusions: These findings suggest that overexpression of pan AKT particularly AKT-2 and p-AKT may be involved with OSCC carcinogenesis and post-transcriptional modification of the expression of AKT isoforms in OSCC may occur. In addition, OSCC cells may undergo epithelial-mesenchymal transition since their epithelial maker (E-cadherin) was reduced whereas their mesenchymal maker (vimentin) was increased.

Antiplasmodial and Immunomodulating Activity of Some Sudanese Herbal Medicine with emphasis on Pristimerin as Antiplasmodial and Antileishmanial Agent

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Background: The Sudan is being the largest country in Africa, covering an area of one million square miles with the differentiation of a semi-arid climatic conditions, where most people in rural areas rely on traditional medicine for the treatment of many infectious diseases.

Objectives: WHO has recently advocated the use of traditional medicine where appropriate health services become inaccessible, therefore, the study aims to investigate the potential antiplasmodial, antileishmanial activity of some medicinal plants and to detect their effect on human lymphocytes proliferation which may imply the ability to potentiate the human immune system.

Material and methods: Forty-nine plant parts representing 26 species from 15 families were extracted and screened for their activity on chloroquine sensitive strains 3D7 and Dd2. Plants were collected according to their traditional use and / or to their taxonomical affiliation to their families that had been reported to have antimalarial activity.

Results: Thirty-four methanol extracts (6%) exhibited significant activity against 3D7 with IC50 values 2.50 µg/ml, whereas twenty-one extract (37%) showed antiplasmodial activity on Dd2 with IC50 values ≤ 50 µg/ml. On the other hand, thirteen extracts (22%) and ten extracts (18%) only showed an activity with IC50, values ≤ 5 µg/ml on 3D7 and Dd2, respectively. Human lymphocytes treated with the most of extracts demonstrated a minimum level of toxic inhibitory effect at concentration ≤ 100µg, whereas Sonchus oleraceus, Balearica aegyptiaca, Acacia nilotica, and A. senegalensis enhanced lymphocytes proliferation. Bioactively directed fractionation of the chloroform extract of the root bark of Meytenia aegyptiaca resulted in the isolation and characterization of the quinonemethide triterpene, (20α,23α,24R)-25-hydroxy-24-oxo-olean-11,13(18)-diene. The structure was elucidated by spectroscopic techniques. The in vitro antiplasmodial activity of the isolated compound against chloroquine-resistant strain (Dd2) of Plasmodium falciparum was IC50 = 0.5µg/ml and its in vitro antileishmanial activity performed on promastigotes of Leishmania major was IC50 = 6.8 ± 0.8 µg/ml while the cytotoxicity on lymphocyte proliferation model was detected at EC50 = 6.8 ± 0.8 µg/ml.

Conclusion: The promising response of Acacia nilotica and Meytenia aegyptiaca accelerates the formulation of some Sudanese plants used in traditional medicine possess a potent antimalarial action with minor effects of lymphocytes proliferation. These plants have been subjected to long-term clinical trials in folk medicine and hence we propose that these plants should be further investigated.

All abstracts are listed in alphabetical order of the presenting author.
Can Erythropoietin be used to prevent brain injury in African Children with Cerebral Malaria?

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Background: Cerebral malaria is associated with high mortality and long-term neuro-cognitive deficits. Erythropoietin has shown neuro-protective properties in different neurological disorders. We hypothesized that the outcome of cerebral malaria is modified by the responses of this cytokine to hypoxia and that high plasma and cerebrospinal fluid (CSF) levels would prevent neurological damage. Aim: Determine the relationship between plasma and CSF erythropoietin and the outcome of cerebral malaria in African children.

Methods: We measured erythropoietin in paired samples of plasma and CSF of 124 children with cerebral malaria. Patients were categorized into 3: 76 without deficits, 32 with deficits and 16 who died. The median (IQR) plasma erythropoietin were compared. Logistic regression models were used to identify risk and protective factors associated with sequelae.

Results: The median (IQR) plasma concentrations of erythropoietin were 123(29-1,729)U/L, 184(23-884)U/L and 276(96-1,852)U/L in children who died, survived with and without sequelae respectively. Conditional logistic regression analysis matching the 32 patients with sequelae to 64 patients without sequelae stratified with and without sequela resulted in 64 patients. Both erythropoietin levels were associated with increased risk of neurological deficits. 2) The age-dependent erythropoietin response to anemia and the age-dependent protective effect may influence the clinical epidemiology of cerebral malaria. 3) These data suggest further study of erythropoietin as an adjuvant therapy in cerebral malaria.

Conclusions: Adhesion of buds to the mother membrane before they become free vesicles. Attractive interaction between membranes and suppress microvesiculation by adhesion of buds to the mother membrane before they become free vesicles.
Molecular investigation of drug resistant Neisseria gonorrhoeae clinical isolates

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Background: The introduction of new adequate methods for correct species identification and susceptibility testing of N. gonorrhoeae (NG) remains an actual problem due to sexual transmitted diseases monitoring. Aims: 1) to show the suitability of the MALDI Biotyper, a system based on mass spectrometry (MS) profiling, for NG species identification, 2) to determine known genetic markers of penicillin (PER) resistance and fluoroquinolones (FQ) resistance in gonococi, 3) to study the relation between genetic markers of drug resistance and the susceptibility profile of clinical strains.

Methods: This study included NG clinical isolates collected from different regions of Russia. Susceptibility testing to PEN, TET and FQ was performed by the agar dilution method according to CLSI. Single colonies of fresh bacterial cultures were tested by the MALDI Biotyper system (www bruker.com) using a Microflex LT MALDI-TOF mass spectrometer. Total genomic DNA from NG strains was isolated using the ‘DNA express’ kit (Lytech Ltd, Russia). The presence of bla and tet(M) genes was analyzed by PCR. Mutations in NG genes (rpsJ, porA, penA, porB, parC, mtrR, norM) associated with drug resistance were detected by primer identification and susceptibility testing of clinical isolates.

Results: Totally, 293 bacterial isolates previously identified as NG were investigated. Susceptibility levels to PEN, TET and FQ were found to be 26 %, 36 %, and 54 %, respectively. For 280 samples the MS profiles were matched to strain NG ATCC 45615. The other MS profiles (13) were similar to each other but different from NG as well as from N. meningitidis, non-pathogenic Neisseria and further 161 different microorganisms stored in the MALDI Biotyper library. If other MS RNA sequencing referred them as unknown species from the genus Ralstonia. The distribution of genetic drug resistance markers was studied for 19 species that belong to 11 different groups of NG strains. Their positive predictive value was found to be different for FQ (90%), PEN (91%) and TET (82%).

Conclusions: 1) The MALDI Biotyper system is a highly suitable tool for the correct NG species identification, 2) the surveillance of genetic markers may be useful for NG monitoring.

Abstracts
Pathophysiological Approach against Resistant Bacteria-causing Infectious Diseases; from Autopsy Findings to Clinical Applications –

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Background: Autopsy is a powerful tool for analyzing the cause of a patient’s death, progression of the disease, and the therapeutic response. However, it is difficult to identify the bacterial characteristics using morphological analysis only, especially in cases of resistant-bacterial strains.

Methods: To elucidate the pathogenesis of resistant bacteria-causing infectious diseases, intrapulmonary sputum was harvested by directly inserting a swab into a resected lung at autopsy, and the bacterial composition was analyzed using both pathological and microbiological techniques from 15 patients with hematological malignancies, and the results were compared with those from 25 patients with other medical and surgical diseases. Then, we established an in vivo infection model to investigate kinetics of pathogens or effects of antimicrobials on slide sections by using autoradiography methods.

Results: Among the 54 bacteria strains isolated from the 40 patients, multi-drug resistant strains were significantly more prevalent in hematological group than in other diseases (16/21 vs. 15/31, p=0.002). Es. faecium was preferentially isolated from the hematological patients, whereas the methicillin-resistant S. aureus was predominantly found in the non-hematological group. Even coagulase-negative S. epidermidis strains in hematological diseases may be diagnosed as causative bacteria of pneumonia by both pathological and microbiological techniques.

In mice or rats infected bacteria or fungi, the control tests at level of therapeutic limits, has demonstrated the more complexity between the ace no coumarol and digoxin TCC could be explained through the different pharmacokinetic mechanisms. 2) The acenocoumarol TCC, requiring two control tests at level of therapeutic limits, has more internationally normalized ratio (INR) determinations in the therapeutic range (INR=2-3) and with a normal distribution of INR values according to the Kortuis and asymmetric coefficient, were selected. For group 2 (N=28), eighty-eight patients were considered with digoxin resistant results with normal distribution comprised from 0.8 to 2 ng/mL and borderline values.

Conclusions: Traditional pathological approach such as autopsy would extend a further analytical tool of resistant-bacteria causing infectious diseases in combination with microbiological or radiological techniques. Our new animal model could also contribute to develop new antimicrobials as well as clinical applications.

Therapeutic Control Chart As A Tool To Aid Drug Monitoring. A Comparison Between The Aacenocoumarol And Digoxin Laboratory Control.

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Background: Retrospective data of patients under oral anticoagulant (OA) and digoxin therapy were used to estimate a reference change using a statistical method. The cumulative probability allow us to obtain if for two consecutive measurements with a p ≤ 0.05, preparing a therapeutic control chart (TCC). The present study was conducted to evaluate the statistical and clinical differences between OA and digoxin compounds in identical pathological backgrounds on the pathological sections (R-s figure represents that the depiction of RI compounds on Aspergillus on slide sections using both pathological and autoradiography techniques).

Conclusions: Traditional pathological approach such as autopsy would extend a further analytical tool of resistant-bacteria causing infectious diseases in combination with microbiological or radiological techniques. Our new animal model could also contribute to develop new antimicrobials as well as clinical applications.

Reduction Of Surgical Site Infections During Paediatric Cardiac Surgery

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Background: Surgical site infections (SSIs) are a substantial cause of morbidity, mortality, and of decreased costs among hospitalized patients. Patients who develop SSIs are up to 60% more likely to spend time in the intensive care unit, 5 times more likely to be readmitted to the hospital and twice more likely to die. SSI are present in patients without SSIs. (1) The objective of this study was to compare the efficacy of a 100 mg/kg dose of fullerenol administered 30 min before surgery with a control group.

Methods: Our new animal model is able to detect any potential protective effects of fullerenol on Dox-induced liver toxicity. The in vivo results (Roy et al. 1994) showed that treatment with 100 µg/kg of Dox alone caused significant changes in the serum levels of ALT, AST, LDH and α-HBDH, as well as in the levels of MDA, GSH, GSHP, TASS, GR, CAT, and SOD in the liver tissue. These effects were drastically reduced for all investigated parameters by pre-treatment with fullerenol, although not for the MDA and GSH level. On the other hand, the human hepatocellular carcinoma (HePG2) cell line was continuously treated with fullerenol for 12, 24, 48 and 96 h, at concentrations of 10 and 44 ng/mL. With the aim of evaluating the modulating activity of fullerenol on Dox-induced hepatotoxicity, the cell line was concurrently treated with Dex (1 µL, 0.5 µL) and fullerenol (10 ng/mL), respectively. The cells were treated with 5 µg Dox along with the fullerenol, a significant development of cell viability during the entire period of observation can be seen. It was concluded that fullerenol has cytotoxic effects on HepG2 by itself, but that the oxidative stress is too high, the cytotoxic effects of fullerenol are overcome by its protective role as a strong antioxidant compound. Damage to the heart muscle after Dox administration was also confirmed by changes in the ultra-structural pathology results and SOD, MDA, CAT, GSSG, GR, and TASS levels, as well as a potential cardioprotective influence of fullerenol as a pre-treatment agent for Dox therapy in the acute phase. Fullerenol itself, in a dose of 100 mg/kg, did not affect the heart injury in rats with liver cancer. The presented results suggested that fullerenol might be a potential cardioprotector in Dox-treated individuals.

Conclusions: The key benefit of fullerenol, in contrast to other known antioxidants, is its dual function as radio-protector and organo-protector during the anticancer therapy (radio-chemo) and human trails. Moreover, there is a need to carry out further studies, including a chronic investigation in animals (this study was done and will be also presented) and human trials.

Materials and Methods: We collected data for 27 children age range (10 days-16 years, mean 18 months), undergoing cardiac procedures. The following data was collected: shower/bath on day of surgery, for SSIs. The median time of our antibiotic administration falls within the desired range of 60 minutes from incision time, however we have noticed and extreme variations among different operators.

Results:

Conclusions: The data collection on antibiotic timing is part of a project to reduce SSIs. Other interventions include preoperative washing on the ward, and improved ground surveillance. Currently the median time of our antibiotic administration falls within the desired range of 60 minutes from incision time, however we have noticed and extreme variations among different operators.

(2) Brazier DW, Houck P. Antimicrobial Prophylaxis for Surgery: An Advisory Statement from the National Surgical Infection Prevention Project Clinical Infectious Diseases 2004: 38-1705-15
Insulin Resistance: Between Myth And Reality

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Background: Almost invariably, during the last two decades it was stated that type 2 diabetes appears as a consequence of increased peripheral insulin resistance (considered to be the primordial factor), associated with a beta cell defect (considered to be secondary both chronologically and as pathogenic importance). There are perhaps few diabetes cases that evolve with a normal beta cell mass. Leaving behind the actual definition/classification of diabetes, we state that the diabetic syndrome is unitary by the decrease of the beta cell mass, considered to be a sine qua non condition for the decomposition of blood glucose regulation. The decrease of the beta cell mass is explained either by an increased apoptosis or by a decreased regeneration or by an association of both mechanisms. Even if the studies of islets obtained from different diabetes animal models indicated an important capacity of regeneration, however, in human diabetes during adult life this regeneration seems to be quasi-inexistent and thus incapable to compensate an increased apoptosis. The cause of increased apoptosis in diabetes could be related to the secretory beta cell dysfunction expressed by the increase of proinsulin and pro-amyl insulin incompletely processed in the endoplasmic reticulum of these cells. Both increased proinsulin and the amylid transformation of amylin could trigger the pro-apoptotic beta cell mechanisms. The decrease of the beta cell mass is usually slow and blood glucose decomposition appears only when >50% of the initial beta cell mass is destroyed. Increased proinsulin can interfere with beta cell regeneration while the biotidocin transformation of amylin can lead to increased beta cell apoptosis mediated by the endoplasmic reticulum. Genetic studies based on the classical candidate gene method in diabetic animal models and human studies based on the Genome-Wide Scan (GWS) techniques, managed to identify a dozen genes involved in T2DM pathogenesis, almost all being somehow related to beta cell function. The assidious investigation of peripheral insulin resistance genes has a predictably failure since an abstract concept based on mathematical equations (as is insulin-resistance) cannot be located in the real human genome. Since the increasing prevalence of diabetes mellitus has to be explained by the intervention of some environmental factors (increase of caloric intake, especially of animal lipids and decrease of physical activity), both influence the transcription of some genes that are involved in the genetic factor (rather epigenetic) involved to the genetically determined limits of the complex mechanisms that ensure the energetic homeostasis of the human body. The constant survival of fuels from the human energetic system raise problems of adaptation that were not encoded in the original genome. Include of insulin resistance in this disorder seems to be improper.

Conclusions: Peripheral insulin-resistance exists and can be well illustrated by the absence of insulin receptors in the rare forms of extreme insulin-resistance with a well-defined genetic basis. The absence of a related mechanism in type 2 diabetes explains the rhetoric question of Flores J.C. form a recent review (Diabetologia 51:1105-1110, 2018): "Where are the insulin resistance genes?"

Synthesis and Evaluation of Highly Potent Antimicrobial Chromanyl-1,2,4-dithiazoles

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Background: A large number of heterocycles of natural and synthetic origin exhibit valuable antibacterial activity. However, they include drugs such as fluconazole, ketocanazole. Recently, dithiazoles, have attracted considerable interest due their high fungistaticity and of other related 1,2-dithia-heterocycles. On the other hand, a number of antibacterial derivative insulin resistance genes has a predictably failure since an abstract concept based on mathematical equations (as is insulin-resistance) cannot be located in the real human genome. Since the increasing prevalence of diabetes mellitus has to be explained by the intervention of some environmental factors (increase of caloric intake, especially of animal lipids and decrease of physical activity), both influence the transcription of some genes that are involved in the genetic factor (rather epigenetic) involved to the genetically determined limits of the complex mechanisms that ensure the energetic homeostasis of the human body. The constant survival of fuels from the human energetic system raise problems of adaptation that were not encoded in the original genome. Include of insulin resistance in this disorder seems to be improper.

Results: Substituted 3-formylchromen-4-one (1a-f) were reacted with two equivalents of thiocarboanhydride and various aromatic amines leading to 3E-(Phenylo)xyl-[1,2,4]-dithiazol-3-[x]-xylchromen-4-one (3a-f) in high yields.

These chromanyl-1,2,4-dithiazoles (3a-f) were evaluated in vitro for antifungal and antibacterial activities. Antibacterial activities (percentage growth inhibition, MIC) were determined on G. aeruginosa and G. vulva bacterial strains i.e., E.Coli, Pseudomonas aeruginosa, Staphylococcus aureus, Shigella flexneri and Staphylococcus coagulase using cefuroxim and chloramphenicol as positive controls. Compounds 3a,d,e,f show very good antibacterial activity. Similarly, the antifungal activities of T-91825 were determined by using turbidimetry method on Aspangillus niger, Geotrichum candidum, Candida albicans and Candida tropicalis employing fluconazole as positive control. Some of the compounds display very high antifungal activity (MIC 5 μM). fluconazole showed MIC 9 μM under similar conditions.

Results: Compounds 3a,d,e,f shows very good antibacterial activity and compounds 3b,f,d have high antifungal activity. In general, compound bearing FCI substituents on chromene ring displayed high antifungal activity. These useful leads can be evaluated for toxicity and developed further

New Features Of Antidepressants Drugs - Modification Of Histamine Kinetics

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Background: Antidepressants drugs (AD) exert their effects by affecting different targets. Detailed insight into the mode of action of AD could lead not only to better understanding their pharmacological effects but also to improve or expand current utilization of these drugs in clinical practice. Our studies were focused on the effects of AD, mainly amitryptiline, on histamine kinetics in experimental animals. Methods: Different types of in vivo and in vitro studies were performed, using cat, rat and guinea pig. Animals were pre-treated with amitryptiline or other AD, given intraperitoneally. In order to investigate effects on histamine kinetics animals received histamine liberator (compound 48/80) or i.v. injection of histamine and we followed plasma and/or blood histamine concentrations. In addition, the ability of amitryptiline to interfere in histamin metabolism was studied by following effects on the two main histamine degrading enzymes, diamino oxidase (DAO) and histamine-N-methyltransferase (HNMT) measured in rat and guinea pig tissues.

Results: AD interfere with histamine system through different mechanisms. Tricylic AD inhibit histamine release and change plasma histamine kinetics after its secretion induced by histamine liberator in the rat. Amitryptiline and other types of AD also significantly lower the increase of plasma histamine levels induced by the injection of histamine and they change the pharmacokinetic profile of the amine in feline and rat plasma and blood. Amitryptiline decreases the rate of DAO release into plasma after the hepamin activation in guine pig. It also increases DAO and HNMT mRNA expression as well as the activity of both enzymes in guinea pig tissues while in the rat it does not affect DAO activity. In vitro studies indicate that amitryptiline change the activity of both histamine degrading enzymes in a concentration- and amphetamines-dependent manner.

Conclusion: Inhibition of histamine release and increased capacity of histamine degrading enzymes in tissues renders lower concentrations of histamine in the tissues which could inhibit the development of allergic/inflammatory response. Rational taking advantage of the growing knowledge on pharmacological effects of amitryptiline and other antidepressants could enrich their use in clinical practice.
Title: Caffeine Sets The Brain's Excitability By Priming The Activation Of The Endogenous Cannabinoid System

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Caffeine is the world's most popular psychoactive drug and stimulant. Caffeine affects vigilance, attention, mood and arousal, and may facilitate attentiveness and alertness. However, the neurobiological mechanisms by which caffeine primes the brain's excitability remain largely unknown. Caffeine dependence, which is thought to be a form of addiction, has been associated with altered expression of the endogenous cannabinoid system (eCB). In this study, we assessed the effects of caffeine on endocannabinoid signaling and the role of eCB in the regulation of neuronal excitability. We found that caffeine potently activates the eCB system, leading to increased expression of endocannabinoid receptor (CB1) and endocannabinoid levels in the brain. These findings suggest that caffeine primes the brain's excitability by modulating eCB signaling, which could have implications for the development of new therapeutic strategies for the treatment of addiction and related disorders. Further studies are needed to understand the mechanisms by which caffeine primes the brain's excitability and the role of eCB in this process.
A Series Of Antibacterial Proteins Made From Inactive Cyt-Like ORF Of Bacillus Thuringiensis Subsp. Israelensis Using The Microgene Polymerization Reaction

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Background: Insecticidal crystal proteins of Bacillus thuringiensis belong to two unrelated toxin families: receptors-specific Cry against insects and Cyt that lyse a broad range of cells, bacteria included, via direct binding to phospholipids. A new cytolysin gene (cyt1Ca) encoding a 60 kDa Open Reading Frame, has recently been discovered in B. thuringiensis subsp. israelensis. Neither bacterial nor viral activity of cyt1Ca expressed in Escherichia coli nor hemolytic effect of His-tagged purified Cyt1Ca has been observed.

Results: In an attempt to endow inactive Cyt1Ca with Cyt1Aa-like antibacterial activity, two amino acids were replaced by QuickChange mutagenesis, E117V and N125A, so as to raise the hydrophobicity of the corresponding region, considered being the membrane-active motif. Serendipitously, the primers used for QuickChange mutagenesis displayed the intrinsic ability to expand into multiple head-to-tail tandem-repeats in the so-called Microgene Polymerization Reaction (MPR). The clones thus obtained include varying lengths of multiple repeats of the amino acid sequence VIE(LKSLLGI)6, corresponding to head-to-tail polymerization of the primer, translated in frame with Cyt1Ca. These versions of Cyt1Ca caused instant arrest in biomass growth and decreased viability upon exposure in E. coli. Multiple insertions into the polypeptide of the non-mutated motif VIE(LKSLLGI)6 were also lethal. To expose toxicity of the latter motif in the original Cyt1Ca, Cyt1Ca was appropriately truncated.

Conclusions: 1) The toxicity of the above motif is ascribed to its amphiphilic nature; toxicity was not displayed in the original Cyt1Ca because of possible motif sequestration by other parts of the protein due to its inherent folding, which may provide a safety mechanism to protect the host bacterium. 2) Combination of MPR with QuickChange can thus be exploited to design and synthetize polypeptides with antibacterial motifs, multiplied within the frame of a given protein. Supported by an Eshkol Scholarship (to MI) and BSF Grant (to AZ).
The three NOS isoforms contain unique sequences that modulate electron transfer: the calmodulin-binding sequence, the C-terminal extension, and the autoregulatory loop in the FMN-binding module of the reductase domain. In the present studies, we have transferred the control conferred by the C-terminus of NOS to NADPH-cytochrome P450 oxidoreductase (CYPOR), which does not contain any of these regulatory elements. The effect of the addition of the C-terminal sequences, specific for each isoform of NOS (21-mer, 33-mer, and 42-mer for iNOS, nNOS, and eNOS, respectively), on the catalytic activity and properties of CYPOR was determined. The aim was to ascertain the possible evolutionary origin of NOS and to address the effect of new peptide recruitment on the development of new functions for CYPOR. Compared to the soluble CYPOR construct to which each of the C-termini was attached by genetic engineering, CYPOR-iNOS (+ iNOS 21-mer) was ~20% inhibited, CYPOR-nNOS (+ nNOS 33-mer) was ~26% inhibited and CYPOR-eNOS (+42-mer) was ~42% inhibited. While similar reduction in 2,6-dichlorophenolindophenol activity was obtained, ferricyanide reduction was affected much less to negligibly. In addition examination of the kinetic constants showed no significant changes in $K_v$ for NADPH (1.88 ± 0.49 to 2.55 ± 0.48 $\mu$M) at 100$\mu$M cytochrome $c$ or for cytochrome c (19.22 ± 2.13 to 27.77 ± 2.43 $\mu$M) at 50$\mu$M reduced flavin content of the constructs. However, the reduction of molecular $O_2$ was increased by the addition of C-terminal sequences, suggesting a shift in the rate-limiting step caused by interference of electron flow between FAD and FMN by the C-terminal extension of the C-terminus over the FAD-FMN interface in the NOS isoform structures. This conclusion has been supported by the published structures of CYPOR and of the nNOS reductase.

The modulation of CYPOR by the addition of the NOS C-terminus is also supported by flavin reoxidation and fluorescence-quenching studies and antibody recognition of the C-terminal extension. These experiments support the origin of the NOS enzymes from modules consisting of a heme domain and CYPOR or ferredoxin-NADP+ reductase- and flavodoxin-like subdomains that constitute CYPOR, followed by further recruitment of smaller modulating elements into the flavin-binding domains.

Supported by project MZO 00064165

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mg/ml) for 2 min, cAMP levels increased 82–190%, improve efficacy of augmentation therapy and to design novel therapeutic drug
dependent PKA; 2) Elucidation of the pleitropic functions of AAT may help to
Artemisinin derivatives are also reported to inhibit the angiogenesis which is required by tumor cells to get the oxygen and nutrients. Artemisinin derivatives significantly inhibit angiogenesis in a dose-dependent manner. Recently clinically relevant anti-tumor effects were clearly demonstrated in man.

Is Levodopa The Magic Bullet For Parkinson's Disease?

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Background: Much progress has been made since James Parkinson first described the disease in 1817, but Parkinson’s disease (PD) continues to be one of the most common causes of disability, particularly among the elderly. Our understanding of the etiopathogenesis of PD has improved markedly with the discovery of genetic causes and with growing knowledge of mechanisms underlying neurodegeneration in the substantia nigra and resulting depletion of dopamine and other neurotransmitters (Pan et al. Brain 2008;131:1969-78). Dramatic improvement in the cardinal signs of PD, tremor, rigidity, akinesia, and postural instability (TRAP) and other motor symptoms, in response to levodopa has been recognized since 1961, when Birkmayer and Hornykiewicz (Wien Klin Wochenschr 1961;73:787-8) first treated parkinsonian patients with the dopamine precursor.

Methods: Evidence-based literature on the efficacy and safety of levodopa was critically reviewed and analyzed in the context of long-term experience. Videos of patients treated with levodopa will be illustrated to indicate its efficacy and motor complications.

Results: The therapeutic options for patients with PD have been expanding with the introduction of dopamine agonists, MAO-B inhibitors, and other medical and surgical strategies, but levodopa continues to be the “gold standard” and is considered to be the most effective drug in the symptomatic treatment of PD. Psychiatric and motor complications, including fluctuations and dyskinesias, can be managed effectively until the advanced stages of the disease. There is no evidence of levodopa-related neurotoxicity from in vivo studies or long-term clinical experience.

Conclusions: Levodopa is not only the most effective drug in the treatment of PD, but it also exemplifies its introduction revolutionized treatment of neurodegenerative disorders and transformed Neurology from a primarily a diagnostic specialty to a therapeutic discipline and as such it may be considered “the magic bullet” in Neurology. Despite its extraordinary impact on the quality of life of patients with PD, there are, however, many limitations to levodopa, including the various acute and chronic complications and its lack of efficacy in certain “ axial” motor signs, particularly freezing of gait and postural instability, and in most non-motor symptoms associated with PD, such as behavioral, cognitive, sensory, autonomic and sleep disorders (Jankovic J. J. Neurol Neurosurg Psychiat 1985;48:611-7).

Sulfasalazine Revisited: A Multi-Targeted Magic Bullet

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Background: Sulfasalazine (SSZ) is commonly prescribed for the treatment of patients with chronic inflammatory diseases such as those with rheumatoid arthritis (RA). Here we report on the novel mechanisms of action of SSZ, SSZ-drug interactions with the folate antagonist methotrexate (MTX), and mechanisms of acquired resistance to SSZ.

Methods: In vitro cell line model systems of immune effector cells (T-cells, NK cells) and macrophages or monocytes, which are involved in the pathogenesis of rheumatoid arthritis were used to evaluate anti-inflammatory properties of SSZ after short-term drug administration or after chronic exposure to stepwise increasing concentrations of SSZ, which provoked cellular resistance.

Results: SSZ proved to be a potent inhibitor (IC50: 0.55 mM) of the production of pro-inflammatory cytokine tumor necrosis factor α via inhibition of the nuclear transcription factor NF-κB. This capacity was 3-fold reduced for cells with acquired resistance to SSZ. The mechanism of SSZ resistance was elucidated in the cell line 721 that is highly sensitive to SSZ. Several studies suggested that SSZ was a potent, non-competitive inhibitor (IC50: 0.3 mM) of the Reduced Folate Carrier (RFC), the dominant transporter for the cellular uptake of MTX and natural folates. Concurrently, this SSZ-RFC interaction provokes an intracellular folate depletion that could further enhance the therapeutic effect of MTX in SSZ-MTX drug combinations, but only when SSZ administration precedes and is spaced in time from MTX administration. Finally, chronic exposure of cells to stepwise increasing concentrations of SSZ markedly increased the cellular sensitivity for the glucocorticoid (GC) drugs prednisolone and dexamethasone. In fact, after SSZ exposure, primary GC-sensitive T cells displayed 10-20 fold greater sensitivity to GCs, while primary GC-resistant macrophage cells resumed full GC-sensitivity due to a greatly enhanced upregulation and stabilization of the GC-receptor, facilitating enhanced GC-induced apoptosis.

Conclusions: SSZ not only elicits potent anti-inflammatory activity as a direct inhibitor of the NF-κB-pro-inflammatory signaling pathway, by targeting other cellular processes (a.o. folate and glucocorticoid metabolism), SSZ allows a rational utilization in drug combinations.
A New Physiological Role for Dopamine and its Transporter in the Pituitary: Induction of Prolactin Cells Apoptosis at Weaning.

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Background: The rat pituitary gland, cessation of lactation causes a massive loss of prolactin (PRL) cells, eliminating the surplus of cells coming from previous proliferation occurring during pregnancy and lactation. The factors and mechanisms involved in this phenomenon were unknown, but our study provides the first evidences that dopamine (DA) plays a key role in this process.

Methods: We tested the pro-apoptotic effect of DA on pituitary primary cells from virgin, lactating, and post-lactating rats. By measuring several apoptotic markers as mitochondrial membrane potential loss, caspase-3 activation, and nuclear fragmentation, we show that DA induces apoptosis specifically in PRL cells from post-lactating rats.

Results: Surprisingly, although the DA receptor (D2R) expressed in PRL cells has been linked to inhibition of cell proliferation, and D2R agonists are used in therapy against pituitary tumors, the D2R is not involved in DA-induced-apoptosis in our context. We then determined that this effect was mediated by the DA transporter (DAT), as revealed by a pharmacological study corroborated by detection of the DAT expression exclusively in PRL cells from post-lactating rats.

In the same time, we also observed the expression of tyrosine hydroxylase (TH), the major enzyme of the DA synthesis in post-lactating PRL cells which was accompanied by an increase in DA content in the AP gland of post-lactating as compared to virgin rats. Finally, we observed that cells expressing TH co-expressed DAT and cleared caspase-3.

Conclusions: Finally, we studied in a PRL cell line model (GH3 cells) the DA-induced apoptotic pathway and showed that, as described in the neuronal model, transport of DA induced oxidative stress, leading to stimulation of pro-apoptotic proteins involved in the mitochondrial pathway (Bax, Cytochrome c) and finally to caspase activation.

Conclusions: These findings show that DA may play an important role in lactotroph regression during the post-lactation period by inducing apoptosis. The fact that this process requires DAT and TH expression by lactotrophs themselves suggests that it may be "autocrine" in nature. This mechanism, already described in neuronal model of Parkinson disease, could be the first physiological example of a regulatory expression of DA and DAT to specifically induce apoptosis. A better understanding of the mechanisms inducing these regulations could help us to explain pituitary tumors formation (particularly prolactinoma), no convincing explanation having never been caracterised so far.

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Comparison of the Pharmacodynamics of Imipenem in Patients with Ventilator-Associated Pneumonia following Administration by 2 h or 0.5 h Infusion

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Background: The time that concentrations in plasma are above the MIC (≧MIC) is the pharmacokinetic/pharmacodynamic parameter correlating with the therapeutic efficacy of β-lactam antibiotics. The aim of this study was to compare the ≧MICs of imipenem between administration by a 2 h infusion with a 0.5 h infusion.

Methods: The study was a randomized three-way crossover in nine patients with ventilator-associated pneumonia. Each subject received imipenem in three regimens consecutively: (i) 0.5 h infusion of 0.5 g every 6 h for 24 h; (ii) 2 h infusion of 0.5 g every 6 h for 24 h; and (iii) 2 h infusion of 1 g every 6 h for 24 h.

Results: Following the 0.5 h infusion of 0.5 g of imipenem, the percentages of the ≧MICs of 4, 2, and 1 mg/L were 20.32% ± 9.32%, 44.11% ± 16.45%, and 64.67% ± 20.56% of a 6 h interval, respectively. For the 2 h infusion of 0.5 g of imipenem, the percentages of the ≧MICs of 4, 2, and 1 mg/L were 17.71% ± 19.27%, 53.75% ± 76.54% ± 17.36% of a 6 h interval, respectively. For the 2 h infusion of 1 g of imipenem, the percentages of the ≧MICs of 4, 2, and 1 mg/L were 60.26% ± 23.96%, 77.78% ± 20.11% and 93.35% ± 8.26% of a 6 h interval, respectively.

Conclusions: 1) The 2 h infusions of imipenem resulted in greater ≧MICs than the 0.5 h infusion. 2) For infections caused by pathogens with high MIC, a 2 h infusion of 1 g of imipenem every 6 h can provide plasma concentrations above the MIC of 4 mg/L for 60% of a 6 h interval.
Impact of Cisplatin potentiation by Cytarabine in the 5-FU-CDDP regimen for dismal-prognosis head and neck cancer (HNC) patients; a meta-analysis of 3 focal trials involving 492 patients.

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Further to our randomized study demonstrating response and survival benefit for Cytarabine (CAR) 1000 mg/m2 in the treating Cisplatin (CDDP) in the standard 5-FU-CDDP regimen (Eur J Cancer 2002) in dismal-prognosis HNC patients, we wished to assess if the neoadjuvant preceding radiotherapy with FU-CDDP regimen (83 pts), Cohort 2 CAR-1000-5-FU-CDDP (153 pts) and Cohort 3 CAR-500-5-FU-CDDP (246 patients). All three regimens were applied both in palliative and neoadjuvant setting, the neoadjuvant preceding radiotherapy with 70 Gy. RR and RD rates were assessed on evaluable patient basis and survival on intend-to-treat basis. Statistical analysis included the chi-square test, the log-rank test, determination of the death hazard radio and Cox regression analysis.

Significance was assessed by the t-test with Bonferroni correction. The RR's were significantly higher in CAR-potentiated Cohorts (Cohort 1 44%, Cohort 2 62%, Cohort 3 66%, p<0.003) and RD rates in the standard 5-FU-CDDP Cohort (Cohort 1 43%, Cohort 2 21%, Cohort 3 15%, p<0.01). MDL survival in Cohort 1 was significantly better than in 2 and 3, 11 months. The one and two years survival was for the Cohort 1 26% and 6%, for Cohort 2 42% and 14% and for Cohort 3 58% and 16%. The difference in survival with the CAR of the last tested HR was highly in favor of both CAR-potentiated Cohorts (p<0.0001) with the power of over 95% for p<0.01. Cox regression analysis showed that both performance status, primary tumor location and treatment schedule were significant predictors of survival. The highest impact on survival had the administration of the CAR-potentiated regimens with a death hazard ratios of 0.58 an 0.61 (CI respectively 0.53 and 0.67) determined to standard 5-FU-CDDP regimen. Potentiation of CDDP by CAR improves both RR and survival in dismal-prognosis head and neck cancer patients; the choice of the neoadjuvant regimen prior irradiation is crucial in judging its benefit impact in otherwise dismal-prognosis HNC patients.

ACKNOWLEDGEMENTS:Supported by NIH grant GM059803.

REFERENCES:

Therapeutics: New Baseline Methods For Parameter Updating in Individual Patients

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The MM-USCPACK clinical software for dosage individualization uses nonparametric (NP) pharmacokinetic models. It develops maximally precise dosage targets to hit with minimum weight-based error. It then compiles the Bayesian posterior probability of each support point in the NP model. Those points fitting the patient's data well become more likely, and vice versa. How the predicted serum concentrations on IIV depend on the patient's weight will typically widely represented by support points in the model. If this is not the case, poor parameter updates may be obtained.

1. We have now developed a new hybrid (H) Bayesian approach to such updating. It uses together with conventional maximum a posteriori probability (MAP) Bayesian estimation. However, extra support points are then added in the region of the MAP estimator to augment the population model for the data it may not hit. These points may be a critical set of support points in that area. It also provides a richer and safer approach to optimized NP Bayesian parameter estimators that avoids the classical M1-M2 cleavage dosage design. This new approach is now being implemented in our clinical software.

2. We have also developed a new sequential interacting multiple model (IMM) Bayesian approach to best track and estimate patient parameter distributions as they change in unstable patients who have changing parameter values with their changing clinical status. All current Bayesian updating procedures assume that there is only one set of fixed parameter values that best fit the data. The IMM Bayesian procedure is widely used in aerospace. It permits parameter values to change during the fitting procedure [1].

Results: IMM tracks drug behavior in a simulated changing patient with less than half the effort of the MAP or IMM procedures. It has been incorporated into the MM-USCPACK clinical software. It now has tracked the behavior of gentamicin and vancomycin significantly better than MAP and IMM methods in over 135 unstable post-cardiac surgical patients. Both tools show great promise in optimizing patient care [2].

ACKNOWLEDGEMENTS:Supported by NIH grants GM086968 and EB005803.

REFERENCES:
P2X Purinergic Receptor Modulation Of Excitatory Nociceptive Transmission Involves NMDA Receptors

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Background: The majority of trigeminal small-diameter nociceptive primary afferent fibres innervating cranial tissues terminate in trigeminal subnucleus caudalis (also termed the medullary dorsal horn, MDH). We have previously shown in vivo that central sensitisation of nociceptive neurons in deep MDH laminae can be induced by purinergic (P2X) receptor agonists and blocked by P2X antagonists applied in this area (Chang et al., 2006). Our in vitro studies indicate that P2X agonists act presynaptically to facilitate MDH excitatory neurotransmission (Jennings et al., 2008). This study aimed to test if these P2X receptor processes involve NMDA receptor mechanisms and PKC. These are important as it addresses some of the underlying mechanisms of prolonged neuronal excitation in pain pathways.

Methods: Sprague-Dawley rat pups (9-16 days) were anaesthetised with halothane, decapitated and horizontal slices (250µm) were cut from the caudal brainstem. Whole-cell patch-clamp recordings (voltage clamped at −70mV) were made from MDH neurons.

Results: An increase in excitatory neurotransmission in deep MDH laminae, as reflected in an increase in mEPSC rate, was induced by the ATP analogue α,β-methylene-ATP (2 µM), evoked a concentration response of mEPSC rate and amplitude (n=6; P<0.05) following superfusion of the NMDA antagonist AP5 (40µM). In other brain areas, phosphorylation of NMDA receptors through PKC has been shown to potentiate glutamatergic neurotransmission, so we tested if P2X receptor-mediated central sensitisation in the deep MDH is mediated via NMDA receptors. Furthermore, our preliminary data suggests Protein kinase phosphorylation of NMDA receptors is involved.

Conclusion: These results suggest that P2X receptor-mediated central sensitisation in the deep MDH is mediated via NMDA receptors. Furthermore, our preliminary data suggests Protein kinase phosphorylation of NMDA receptors is involved.

Effect of Fish Oil with Garlic Oil Supplementation on the Anthropometric Measurements, Serum Lipid Profile and Blood Pressure in Women with Hypercholesterolemia and Hypertension

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Background: Elevated levels of low density lipoprotein (LDL) triglycerides (TG), serum lipoprotein(a) (Lp(a)) and low levels of high density lipoprotein (HDL) have been documented as leading risk factors for the development of hypertension and cardiovascular disease (CVD) in Indian women. In view of observed increase in LDL levels with fish oil supplementation (Suppn’), the effect of a combined supplement of fish oil (Mega-3) with garlic oil (PARKLIarchs) on the anthropometric (AM), serum lipid profile (SLP) and blood pressure (BP) levels in women with hypercholesterolemia and hypertension were studied. Aim: To assess the effect of fish oil ( supplemented with garlic oil (GO)) supplement on the AM like body mass index (BMI) waist circumference (WC) and waist:hip ratio (WHR) in Indian women.

Methods: This study included 60 hypercholesterolemic (>200 mg/dl) and hypertensive (>140/90 mmHg) women of which, an equal number (n=30) belonged to the premenopausal group(30-45 yrs.) and the postmenopausal group(46-60 yrs.). The subjects were further subdivided into two groups within each group as control group and test group. The study was conducted for a period of 90 days and with a withdrawal period of 30 days (Test groups) The dosage of fish oil was 600mg per day and garlic oil was 500mg per day (Test groups) in both the biochemical parameters were analyzed using the enzymatic kit methods.

Results: Statistically significant reductions were seen in all the AM, SLP(except HDL) and BP levels of the women in the test groups after 90 days of supplement compared to that of the respective control groups. After the withdrawal period, the hypercholesterolemic and hypertensive effect of P2X with GO was sustained in both the test groups.

Conclusion: The results provide evidence for the role of lipid lowering and antihypertensive capabilities of Fish Oil and Garlic Oil in the management of Hypercholesterolemia and Hypertension.

Development of an Inactivated Rotavirus Vaccine for the Global Immunization Agenda

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Effectiveness of Top2 inhibitor needs to be considered in the context of DNA-histone interaction.

Abstracts

Effect of Fish Oil with Garlic Oil Supplementation on the Anthropometric Measurements, Serum Lipid Profile and Blood Pressure in Women with Hypercholesterolemia and Hypertension

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Conclusion: The results provide evidence for the role of lipid lowering and antihypertensive capabilities of Fish Oil and Garlic Oil in the management of Hypercholesterolemia and Hypertension.
Is Cyclosporine The Magic Bullet In Dermatology?

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The effects of cyclosporine explain the interest in its history. Since the drug of fungal origin with anti-inflammatory properties, low myelotoxicity, and the T-cell immunomodulating effects revolutionized transplantation medicine, it was claimed that serendipity played a significant part in its discovery. Cyclosporine acts primarily during T cell activation by modulating lymphokine production and by reducing the activation and proliferation of T-helper and cytotoxic T cells. These selective effects have been recognized as beneficial in the treatment of dermatologic diseases, and this has particularly revolutionized skin immunointerfering. The only dermatological indication for cyclosporine approved by the US Food and Drug Administration is psoriasis. The authors discuss excellent therapeutic responses reported in patients with other dermatologic diseases: atopic dermatitis, erosive mucosal lichen planus, Behoïd's disease, epidermolysis bullosa acquista, lichen planus and pyoderma gangrenosum. Cyclosporine A can be safely administered when potential toxicities, dosing (3-5 mg/kg), and guidelines are known. Also, highly variable results of systemic cyclosporine in the treatment of severe alopecia areata, have been achieved. The authors of this review following the concepts of lower dosages of systemic cyclosporine (2.5 mg/kg/monthly) and the use of low dose prednisone simultaneously (5 mg/d), developed complete long-duration sustained terminal hair re-growth in universal alopecia areata (chances for cure was less than 1%). Thus, in spite of the tremendous progress in immunologic research, practical therapeutic immune-intervention has yet to reach the level of specificity and precision that is needed, i.e. the “immunologic magic bullet”. However, cyclosporine is a good candidate.
Discovery & Development of Antineoplastic Magic Bullets from Natural Products

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**Background:** Most clinically useful anticancer drugs were discovered from natural products as exemplified in adriamycin, vinblastine, taxol, and camptothecin, etc. Aims: 1) A practical and stereospecific synthesis of novel derivatives of deoxoartemisinin and daumone, a kind of pheromone. 2) In vitro and in vivo testing of antineoplastic activity of the synthesized compounds. 3) Establishment of structure and activity relationship (SAR) to design and provide an anticancer drug candidate.

Previously, we discovered a novel pheromone that postpones aging in worms from *C. elegans* with a laborious, large-scale purification and 3-dimensional structural determination.

**Methods:** This study included synthesis, anticancer activity screening and SAR study including proposed biomechanism of anticancer activity. Most of synthetic efforts have been focused in derivatization at C-12 position of artemisinin resulting acetal-type derivatives. To overcome instability in simulated stomach acidic condition, and recently appearing neurotoxicity of artemisinin and its C-12 acetal derivatives such as arteether, artemether, and artelinic acid, we prepared non-acetal type deoxoartemisinin and its derivatives from artemisinin, a natural product isolated from *A. annua* as potential anticancer drug candidates. Total synthesis of new derivatives of dauer-effect pheromone isolated *C. elegans* were efficiently achieved.

**Results:** Non-acetal C-12, 13 derivatives, (+)-deoxoartelinic acid and dimers of (+)-deoxoartemisinin were synthesized either from naturally occurring artemisinic acid or directly from artemisinin via a short and regiospecific process. Some of its novel derivatives show comparable antitumor activities to those of clinically useful drugs.

A stereospecific, 10 step synthesis of pheromone was successfully achieved starting from commercially available rhamnose. Both isolated and synthetic daumone induce the morphological changes that accompany worm hibernation. The detail of stereospecific synthesis and the antineoplastic activities of (+)-deoxoartemisinin, its C-12 and 13 derivatives, natural plakortolide and novel anticancer derivatives of daumone along with the structure-activity relationship will be presented. In vitro antineoplastic screening of derivatives of deoxoartemisinin and daumone against human cancer cell lines (ovary, lung, brain, colon) showed potential anticancer activities.

**Conclusions:** 1) The non-acetal type and anticancer derivatives of deoxoartemisinin showed acid stability, and may overcome neurotoxicity. 2) These results may shed light on the discovery of antineoplastic magic bullets derived from natural products and a signaling pathway thought to be similar to those that lead to aging and obesity in humans. 3) Trimers of deoxoartemisinin and daumone against human cancer cell lines showed potential anticancer activities comparable to that of clinically useful anticancer drugs.
Abstracts


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Background: 'Magic bullets' for ocular diseases have primarily focused on the treatment of neovascularization and the prevention of secondary cataracts. Patients with macular degeneration are intravitreally injected with anti-VEGF antibodies to retard angiogenesis while anti-neoplastic agents are being investigated for the prevention of post-surgical lens cell proliferation. Combretastatin A-4 (CA4P) is a vascular targeting agent that destroys rapidly growing tumor capillaries. In mouse eyes, CA4P also suppresses retinal neovascularization that occurs within days to weeks following laser-induced choroidal neovascularization or genetic overexpression of retinal VEGF. To determine the clinical potential of CA4P to treat neovascularization in diabetics, we have evaluated CA4P in galactose-fed dogs, an animal model that slowly develops proliferative retinopathy similarly to that observed in diabetics.

Methods: Eight beagles fed 30% galactose diet for 90-104 months and 4 age-matched normal beagles were made aphakic so that retinal changes could be easily documented. These were divided into 2 groups composed of 4 galactose-fed and 2 control dogs. Each group received CA4P as either sub-Tenon’s or intravitreal injections. Six weeks later, all dogs received systemic (IV) injections of CA4P. Changes in neovascularization and blood flow were clinically monitored at 2-week intervals by fluorescein angiography and OCT. Protein overlay assays and Western blot analyses were performed using anti-rabbit antibodies. Tissue specific localization of the protein is also performed to know whether raised antibody can detect in situ localization of the isoforms.

Results: Ten region-peptides of the ryanoide receptor of C. elegans (CaRYR) containing 5071 amino acid residues were produced in Nigella sativa (L.) seeds and tested in this study.

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Conclusions: Antibody based protein-function analysis on the ryanoide receptor and troponin isoforms in Caenorhabditis elegans is applicable for determining epitope in functional regions of the protein. 1) Antibody based analysis on molecular interactions allows us more information than those of sequence alignments. This experimental approach is powerful to solve biological function of many accumulated protein sequences and is applicable for determining epitope in functional regions.

Peculiarities of Blood-Brain-Barrier Penetration of Praidoxime

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Background: Organophosphate poisoning in agriculture (millions of cases) and the threat of chemical terrorist attacks require intensive research on antidotes. Organophosphates cause overabundance of acetylcholine at all kinds of its receptors in the central nervous system, and the peripheral. Antidote treatment of victims is possible, the therapy is known by the acronym ‘AFLP®’ (atropine, oxon, oxygen and pralidoxime). Clinical experiments, however, have been disappointing with any one of the presently available antidoxes. Fast and effective disposition and excretion of the organophosphate – aldoxime conjugates (phosphonyloximes, POXs) may have vital importance as POXs play an essential part of anticholinesterase activity, and POXs may be hydrolyzed with endogenous enzymes to give the original organophosphates.

Methods: Lipophilicity of pyridinium aldoximes was determined using silicon (planar chromatography) and in silicon (computer assisted lipophilicity calculations) methods. Distribution/penetration of pyridinium aldoximes were determined using reversed-phase chromatography of blood, CSF (cerebrospinal fluid) and brain samples of rats following intramuscular treatments of the animals. Various brain regions were also dissected.

Results: Both thin-layer chromatography and computer-assisted lipophilicity calculations showed highly polar characteristics of pyridinium aldoximes. Even, pralidoxime and the other N-substituted pyridinium aldoximes do enter the brain and the cerebrospinal fluid. Results indicate that low doses of pralidoxime, K-27, K-48 and K-203 (doses below 10 microM) show higher relative brain and CSF penetration. Considering the various brain parts (frontal cortex, hypothalamin, hippocampus, striatum, etc.) pyridinium aldoximes show an even distribution.

Conclusions: For patients who have been poisoned, infusion with low-concentration pyridinium aldoximes may be preferable to one injection with a high dose.
Novel Immuno- Potency Drugs

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Background: The protective role of the immune system in health and the correlations of its failures as a path of pathogenesis are well clarified. Based on recent studies the immunologic concern in the field of health has been improving. The occurrence of diseases like viral, bacterial and fungal diseases, auto- immune diseases and cancer in turn denotes to immunological problems as natural and acquired immune system defects. Because of critical need to support the immune system we proposed that there is need to design kinds of novel generations of drugs to get such a goal. We aimed with this strategy to support the immune system in a way through which it potentiates itself to fight against pathogenic factors, regulate, modulate or reverse the defects as needed.

Methods: Three different drugs prepared from bacterial and lipid extracts (G2, PC & G2F) that could increase Th-1 cells response depending on the methods of experiments including Delayed type Hypersensitivity (DTH), Lymphocyte Transformation Test (LTT) with PPD and rgp63 cocktails. They prepared in cream and injection forms. The clinical trials Phase II, I performed successfully and also III done in regard with some diseases.

Results: Based on methods of experiments Th-1 response was significant (P<0.01 to P<0.001). Cytogetic and mutagenic studies showed no chromosomal abnormalities. Heating of up to 42% ductal adenocarcinoma of breast cancer in lab rats, with significant increase in survival (P<0.000). Control or cure or prevent after surgery of different cancers in volunteers such as: Leukemia, Breast Cancer, Macroadenoma, Prostate cancer of urinary gland, colorectal cancer, Hepatitis and other types. These drugs controlled and cured (followed up) of 70% of adult and up to 80% of children asthmatics respectively. Complete healing of grade 3-5 of diabetic foot ulcers in some volunteers with resistant to therapy wound types. Newly generated foot ulcers in diabetic patients cured within few days.

Conclusions: We concluded that the formulations mentioned above are optimized compounds of effectiveness with no side effects reported till now. Because of its effectiveness we suppose it as a remedy of next decade. It would be applicable in treatment of chronic diseases, cancers and in all fields required the body to get normal by regulating, modulating and reversing the defects.

A Preparation Of Ni-Lipid Nanoparticles By Combination Of Roll Mill And High Pressure Homogenization, And Stabilization Of The Nanoparticles By Gelatin Solidification —

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Background: Nanotechnology has recently been attracting attention, and establishment of pharmaceutical technologies to micronize drug particles is extremely expected. However, almost all methods use organic solvents in the process of preparing nanoparticles. Accumulation of residual solvents in the body and environmental pollution by liquid waste are matters of concern regarding the use of organic solvents. Thus, we try to prepare nanoparticles without using organic solvents. In addition, we investigate a method to maintain the dispersed condition of the nanoparticles by adding gelatin and solidifying the suspension. Freeze-drying allowed reproduction of the nanoparticle condition. However, this procedure is inconvenient for oral administration due to requiring preparation at the time of use. Simple method substituting for freeze-drying method was desired.

Methods: A 30:100D (weight ratio) of Nipidicene (Ni)-phospholipid (PL) mixture prepared by roll milling was dispersed in water and premixed. Subsequently, the premixed suspension was applied to high-pressure homogenization (HPH). Physical characteristics of Ni-PL mixtures were analyzed by using powder X-ray diffraction. For the in vitro assessment, infrared spectroscopy. Particle size was measured by using light scattering photometer.

Results: The mean particle size of the Ni-PL nanoparticles decreased as the pass number increased, and the size after 40 passes were 55nm, indicating that roll mixing was as effective as ethanol treatment. Ni-specific diffraction peaks of powder X-ray diffraction appeared at 8.2, 16.2, 24.4, and 25.9°. The peaks of Ni-PL mixtures were present at the position of Ni crystals, and no peak shift was induced by interaction with PL, showing that Ni mostly remained as crystal in the PL.

Conclusions: 1) The Ni-PL nanoparticle suspension prepared by combination of roll mill and HPH, indicating that nanoparticles could be prepared without organic solvents. 2) The mean particle sizes were about 55nm before and 24nm after the gelatin solidification, suggesting that gel solidification method is helpful.

Symbiotics: An Important Tool For Preventing Enterocolitis And Promoting Physical Growth In Severely Ill Pediatric Patients —10 Years’ Experience In One Institute

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Background: Pediatric surgical patients often suffer from severe enteritis and it causes sepsis, hepatic failure and malnutrition. In order to disclose the etiology of these complications we investigated the intestinal microbiota of the patients and the effects of probiotics.

Methods: We designed a new symbiotic therapy consisted of bifidobacterium and other lactics such as Streptococci, and galactooligosacharides. Our experience was the first clinical trial of symbiotic therapy in the world. We applied symbiotic therapy to more than 50 pediatric patients who had short bowel syndromes, intestinal functional disorders, severe respiratory distress, and liver dysfunctions with abnormal intestinal microbiota (therapeutic use of symbiotics). Intestinal microbiota and nutritional states were followed before and after the therapy. Recently we administered our symbiotics to neonatal surgical patients who had not yet acquired intestinal microbiota (prophylactic use of symbiotics).

Results: Almost all patients had a quite abnormal intestinal microbiota; decrease of beneficial bacteria and increase of anaerobic bacteria and increase of pathogenic microorganism. After starting the therapy many patients acquired the probiotics dominant intestinal microbiota and it was maintained well during through the treatment course. When the patients recovered from the critically ill states, the intrinsic anerobes increased in the intestine and probiotics suppressed in its number. The nutritional states improved with symbiotic therapy in many patients.

As to prophylactic use of symbiotics, being still preliminary results, it is very effective for severely ill infants to establish anaerobes dominant intestinal microbiota and maintain good nutritional states.

Conclusions: 1) Our symbiotics are safe and effective for pediatric surgical patients with abnormal intestinal microbiota. Probiotics were well resided in the intestine and prevented enteritis, improved intestinal functions and patient's nutritional states (therapeutic use of symbiotics). 2) Early start of symbiotics for neonatal patients is more effective to acquire normal intestinal microbiota and it is recommended for very severe neonatal surgical patients (prophylactic use of symbiotics).
Betaine is found in many microorganisms, plants, and marine animals. Its main physiologic functions are as an organic osmolyte to protect cells under stress and as a source of methyl groups needed for many biochemical pathways. Betaine is also found naturally in many foods and is most highly concentrated in beets, spinach, grain, and shellfish. Strains of enterobacteria that cause urinary tract infections are able to grow in urine with high toxicity. The betaine analogues have antibacterial effect against E. coli strains, but only in presence of an osmotic stress. Analogues of gamma-hydroxybutyrate appear to be prospective drugs for the treatment of circulatory complications of sepsis. Betaine is used by cells to defend against changes in osmolality. It is suggested that there are relationships among betaine, osmolality and coccidioidomycosis. The chemotaxis of monocytes toward chemotactic factors released by heterophils was increased by betaine. Increased chemotaxis of monocytes and NO release by macrophages may explain the decreased intestinal pathology but increased leukocyte numbers that were observed when betaine was fed during a Cocci infection. Urine has long been known to inhibit the activity of ammolygocides against urinary tract pathogens. Glycine betaine which is present in urine confers resistance against high osmolality to Gram-negative organisms. The betaines in urine permit the expression of increased resistance to ammolygocides in concentrated urine. Hepatitis C virus (HCV) infection is an important cause of chronic liver disease. Standard interferon-alpha (pegIFNa) combined with ribavirin, results in a sustained response rate in approximately half of patients. It has been shown that treating cells with S-adenosyl-L-methionine (AdoMet) and betaine could restore STAT1 methylation and improve IFNa signaling. Furthermore, the antiviral effect of IFNa in cell culture could be significantly enhanced by the addition of AdoMet and betaine. In our study, we investigated the protective effect betaine and prednisolone on the level of nitric oxide in sepsis.

**Methods:** To verify the effect of the S-adenosyl-L-methionine (AdoMet) and betaine on the STAT1 methylation, the expression of interferon alpha signaling, we obtained genetic evidence that S-adenosyl-L-methionine (AdoMet) and betaine could restore STAT1 methylation and improve IFNa signaling. Furthermore, the antiviral effect of IFNa in cell culture could be significantly enhanced by the addition of AdoMet and betaine.

**Results:** The average number of revertant colonies per plate treated with PHB was less than double as compared to that of negative control. Also, the PHB did not alter the expression of the proto-oncogenes (p53 and c-myc) and anti-apoptotic genes (bcl-xl and bcl-xs) in this study under the present test conditions.

**Conclusion:** The Salmonella mutagenicity test indicated that the locally produced PHB is non-mutagenic on the strains TA1535, TA1537, TA1538, TA98 and TA100. The total RNA was isolated and reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out for β-actin, p53, c-myc, bcl-xl and bcl-xs genes. The amplified products were run on an agarose gel and then quantified based on the intensity of bands of the different genes.

**Results:** The average number of revertant colonies per plate treated with PHB was less than double as compared to that of negative control. Also, the PHB did not show over or under expression of the genes studied.

**Conclusions:** 1) The Salmonella mutagenicity test indicated that the locally produced PHB is non-mutagenic on the variants of Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100. 2) PHB does not alter the expression of the proto-oncogenes (p53 and c-myc) and anti-apoptotic genes (bcl-xl and bcl-xs) in this study under the present test conditions.
In Vitro Preclinical Studies For A Rational Design Of Cancer Chemotherapy Combinations

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Background: Combination chemotherapy has been used for the treatment of cancer, based upon theoretical advantages and on proven clinical efficacy. In vitro drug combination studies play an important role in designing and optimizing combination protocols. However, the design and analysis of preclinical studies to assess the cytotoxic effects in combinations is complex, and there is no consensus as to the best method, since dose-response curves of anticancer agents are variable. Even the same data are used, the results are often different, depending on the evaluation method used.

Methods: A variety of cancer cell lines were used for drug combination studies. Cells were exposed to two drugs simultaneously (and sequentially). Cell growth inhibition was determined using MTT assay. We used the isobologram method (Steel and Peckham) for studying the cytotoxic effects of drug combinations, since it can cope with a variety of doseresponse curves of anticancer agents. This method is generally stricter for synergism and antagonism than other methods.

Results and Conclusions: 1) Therapeutic benefit was highly dependent on the combination and schedule of administration; 2) The anticancer agents which it was easy to use together (additive or synergistic effects in simultaneous exposure) included alkylating agents, anthracyclines, cisplatin, etoposide, imatinib, irinotecan, and antimetabolites (excluding antifolates). 3) The simultaneous administration of methotrexate and all other agents studied produced antagonistic effects due to the increased production of free metabolites. 4) Similar tendency was observed in peritoneal in combination with cisplatin, gemcitabine, irinotecan, or paclitaxel. 5) Paclitaxel had a tendency to act subadditively when administered simultaneously but additively when administered earlier. 6) These findings are useful for designing clinical trials of combination chemotherapy.

Infection Site Concentration Of Metronidazole (MTZ) And Meropenem (MER) In Patients With Septic Shock (SS)

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Background: Sepsis is a frequent syndrome and cause of mortality in modern intensive care. The inadequate concentration of antimicrobial drugs in site of infection might be one of the therapeutic failure reasons. Microdialysis (MD) is a sampling technique of tissue fluid, allowing protein-free fluid sampling from different tissues. The main purpose of the present work was to elucidate distribution of MTZ and MERO in clinical conditions such as perioperative prophylaxis, SS and severe peritonitis.

Methods: 18 patients were studied: 6 female patients receiving MTZ in perioperative period, 6 male patients in intensive care unit (ICU) receiving MTZ for the treatment of anerobic infection and 6 patients in ICU receiving MERO for the treatment of severe peritonitis associated with SS. Both drugs were given intravenously. MTZ sampled from plasma (PL) and muscle tissue (MT) using MD and MERO from PL and peritoneal cavity (PC) using MD. Samples were taken at predetermined time-points before and after administration of drugs. Drugs' concentration were measure using HPLC with ultraviolet detection (MTZ) and LC-MS/MS (MERO). Pharmacokinetic-pharmacodynamic (PK/PD) modeling were also performed: in vitro with MTZ and using mathematical modeling with MERO.

Results: MTZ mean max concentration (Cmax) in surgical patients and patients with SS was 16.5 and 11.4 mg/L in PL and 7.8 and 8.2 mg/L in MT, respectively. Time over minimal inhibitory concentration (T>MIC) was 23.3 and 36.2 hours for MT of surgical and septic patients, respectively. The ratio of Cmax/MIC was also higher in septic patients being 31.1 and 32.7, respectively. In vitro PK/PD modeling using MTZ concentration from patients with SS showed that time for kill 99.9% of microorganism is between 1 and 3.5 hours after exposure. MERO mean Cmax in PL and PC was 86.1 and 36.8 mg/L, respectively. T>MIC was at least 87% of interbarring interval for MIC 4 mg/L in both PL and PC and was 55 and 43% for MIC 16 mg/L in PL and PC, respectively.

Conclusions: 1) Distribution of MTZ is not significantly different in healthy patients and SS patients. 2) MTZ demonstrates excellent antimicrobial killing 3) Concentration of MERO in PC in case of severe peritonitis is high enough to produce antimicrobial killing.

Authors’ disclosure statement: Concentration of MERO in microdialysates was determined by Lefevre S., Marchand S and Coute W, University of Poitiers, France. The PK/PD analysis for MERO was performed by prof. Sawchuk RJ, University of Minnesota, USA and prof. Coute W, University of Poitiers, France.

Differential Targeting Of Immune Response After Antigen Encounter At Different Mucosal Sites – A Tool For Vaccine Development

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Antigen encounter results in activation of specific lymphocytes, which then leave the site of antigen encounter and enter the blood as plasmablasts. These cells are found only temporarily in the circulation while on their way to home to tissues, where they settle down as the final end-stage B cells, plasma cells. While representing only 1% of all circulating B cells, plasmablasts are the only active effector B cells in the circulation, often identified as cells producing specific antibodies against the recently encountered antigen. Plasmablasts express homing receptors (HR) and chemokine receptors (CCR) that allow them to leave the circulation through the endothelium and enter the tissues. Even if blood takes the cells everywhere in the body, plasmablasts can only enter those tissues where the ligands for their HR and CCR are found. The tissue-specificity of homing results from a differential expression of chemokines and ligands for HR between the tissues. The selection of HR and CCR is imprinted on the activated lymphocytes already at the original site of antigen encounter by dendritic cells. Investigation of homing-associated molecules, the homing profile, on plasmablasts provides information on the targeting of the immune response in the body. Almost all pathogens are encountered at mucosal surfaces. These portals of entry are guarded by the local mucosal immune system. Mucosal immunization is the most effective way to induce mucosal immune responses. The different mucosal sites in the body are regarded to be interconnected with one another via circulating lymphocytes thus constituting a sc. Common Mucosal Immune System (CIMS). Antigen encounter at one mucosal site can elicit an immune response at a distant mucosal site. However, lymphocytes don’t migrate equally to all mucosal sites, but are compartmentalized within the CIMS. Our results show that in hystis, antigen encounter in the intestine, urinary, genital, upper and lower respiratory tract each label the circulating plasmablasts with a characteristic homing profile. As the targeting of the immune response depends on the site of antigen encounter, vaccines should be administered to sites from where the immune response is most effectively targeted to that particular site (intestinal, genitourinary, respiratory tract), where the immune protection is desired the most.

Toxic Effect Of Homocysteine On Nervous And Immune System

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Homocysteine (HC) is a risk factor of neurodegenerative and cardiovascular diseases. In terms of effect of HC and homocysteic acid (HCA, the product of spontaneous oxidation of HC) on NMDA-receptors (Carperet of 984A) toxic effect of HC and HCA on NMDA-receptors on neuronal and immune competent cells was studied. These compounds were found to activate NMDA-receptors in a dose dependent manner (0.1-1 mM) resulting in calcium and reactive oxygen species signal and subsequent activation of MAP kinase. Over-loading of NMDA-receptors with these ligands induces injury process in both neurons and lymphocytes resulting in apoptosis at lower concentrations and necrosis at higher concentrations. Thus hyperhomocysteinaemia induces death of the neuronal and immune competent cells resulting in massive exhaustion of both systems. Prenatal hyperhomocysteinaemia induced by over-loading of pregnant rats with dietary methionine (1 g per kg body weight) resulted in modification of properties of NMDA-receptors and memory and behavioral deficiency. Treatment of these animals with carnosine, natural neuromodulator and antioxidant preserved the pups against toxic effect of hyperhomocysteinaemia preventing loss of body weight and memory deficiency. We have concluded that carnosine may protect metabolic function of neuronal and immune systems against systemic oxidative stress induced by HC.
Factors That Influence The Prevalence Of Drug-Drug Interactions Between Antiretroviral Drugs Prescribed To Patients Of Different Age Groups In A Section Of Private Healthcare Sector In South Africa

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Background: Drug-drug interactions (DDIs) are often a serious complication due to taking multiple medications and an increase in the elderly population. This study was undertaken to assess the impact of age on the occurrence of DDIs in a private healthcare sector.

Methods: The study was conducted at a private healthcare sector in South Africa. The study population comprised of patients aged 12 to 85 years who were prescribed one or more ARVs. The Antiretroviral Drug Interaction Database (ARVID database) was used to assess the occurrence of DDIs.

Results: The study included 215 patients; 107 (50.0%) were women and 108 (50.0%) were men. The median age of the study population was 37 years (range: 12 to 85 years). The most common ARVs prescribed were tenofovir and efavirenz. The overall prevalence of DDIs was 29.7% (64/215). The prevalence of DDIs was highest in patients aged 75 years and older (42.8%). The most common DDIs were those involving antivirals and antibiotics, followed by antivirals and immunosuppressants. The most common drug-drug interactions involved drugs used to treat diabetes, hypertension, and psychiatric conditions.

Conclusions: The prevalence of DDIs is highest in older patients. AGDIs are most common between antivirals and antibiotics, followed by antivirals and immunosuppressants. This highlights the need for increased awareness among healthcare providers regarding the potential for drug-drug interactions in patients prescribed multiple medications.

EMY162 Protein As A Vaccine Candidate To Reduce Level Of Alveolar Hydatid Disease

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Background: Echinococcus multilocularis is a parasitic disease. The life cycle of E. multilocularis generally occurs in foxes and rodents as intermediate hosts. Humans can be infected by accidental ingestion of the parasite eggs from an infected fox, or occasionally from infected dogs or cats. Infection in humans causes alveolar hydatid disease. The disease is a significant public health problem. We attempted to clone cDNA of secretory proteins involved in immune defense systems in order to use them in the control of alveolar hydatid disease.

Method: E. multilocularis (Nemuro strain) were obtained from a dog-cotton rat life cycle maintained at the Hokkaido Institute of Public Health. Immature adult worms were collected on day 20 post-infection from cotton rats experimentally infected with E. multilocularis protoscoleces. Total RNAs from the adult worms were isolated. A cDNA library based on mRNA from adult worms of E. multilocularis was constructed. One of the cDNA clone, emY162, was isolated from this cDNA library. Recombinant emY162 was subcloned, and then recombinant antigen EMY162 was administrated to mice with Freund’s complete adjuvant. Antibody production was assayed by indirect immunofluorescence. Immunohistochemical reactivity was analyzed by Western blot. After the final immunization by the recombinant antigen, parasite eggs were administrated orally for vaccine trial of the recombinant EMY162. The number of alveolar cyst in each mouse was counted.

Results: The putative protein from emY162 cDNA consists of 153 amino acids and has a predicted molecular weight of 17.0 kDa. The amino acid sequences of EMY162 are predicted to have single fibronectin type III-like domain. The emY162 protein was expressed in all four stages (protoscoleces, cultured metacestodes, immature adult worms and mature adult worms). When immunity to recombinant EMY162 was examined, strong IgG immune responses were detected in Western blot. The recombinant EMY162 antigen-specific antibody response showed a polarization toward IgG2 subclass. In addition, the recombinant EMY162 induced a significant level of IgG2a format. The recombinant EMY162 antigen-specific antibody response showed a polarization toward IgG2 subclass. In addition, the recombinant EMY162 induced a significant level of IgG2a format.

Conclusion: 1) The Emy162 protein could target both mucosal and systemic immunity in dogs and humans. 2) The EMY162 protein will help the development of both protection against and diagnosis of alveolar hydatid disease.
Adenosine triphosphate (ATP) is released as an autocrine/paracrine signal from a variety of cells. The present study aimed to clarify the Ca\textsuperscript{2+}-signal pathway involved in the caffeine-inducible transport of ATP from cultured vas deferens smooth muscle cells. The transport of ATP induced by caffeine (3 mM) was almost completely inhibited by nystatin and tetracaine, but not by 2-APB, thus being mediated by nystatin receptor (RyR). The expression of messenger RNA from only RyR-2 was detected in the cells. Furthermore, the induced transport was attenuated by mitochondrial inhibitors, rotenone and oligomycin and by Ca\textsuperscript{2+} channel blockers, rifamycin and NPPB. Increase in Ca\textsuperscript{2+}-signals with fluo-4 and mod-2 caused by caffeine were reduced by tetracaine and oligomycin plus CCCP, respectively. A close spatial relation between the endoplasmic reticulum (ER) and mitochondria was electromicroscopically observed in cells, supporting the existence of a Ca\textsuperscript{2+}-signaling bridge on both the organelles. These results suggest that caffeine stimulates nystatin receptor (RyR-2) and facilitates a Ca\textsuperscript{2+}-signal transducing system from ER to mitochondria, and then, the signal appears to accelerate the ATP synthesis in mitochondria. In addition, the mitochondrial event may lead further cell signaling to the cell membrane and activates Ca\textsuperscript{2+} channels, resulting in the extracellular transport of cytosolic ATP. In the study with MDCK cells, we also provided evidence that such a Ca\textsuperscript{2+}-signaling pathway from ER to mitochondria mediates the transport of ATP induced by adenosine.

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Structure Based Development Of Selective Inhibitors For Individual Cathepsins And Their Medical Applications For Therapeutic Purposes

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Background: Cathespsins (Cath.s) are cysteine proteases in lysosomes. Eleven kinds of cath.s are registered in human genome. Cath.s play an essential role in protein catabolism. Since each cath.s has a different cleavage-domain, different cath.s produce different product from the same protein. Abnormal expressions of cath.s induce special diseases, therefore the specific cath.s are useful for pathogenesis and also for the therapy.

Methods: The study of cath. Inhibitors has been started from the discovery of natural inhibitors from bacteria. One is aliphatic aldehyde derivatives to bind with SH-of cath; leupeptin and antip from Uhrovac. The other is derivatives of epoxy succinic acid (ES), ES-64 by Katunuma and Hanada. However, they inhibited all cath. family. Using these frame inhibitors, we developed the specific inhibitors for individual cath.s, based on their different tertiary structures of their substrate binding pockets using their X-ray crystallography.

Results: 1) Specific cath. Inhibitor design. (a) As the ES derivatives. Cath.B specific inhibitors having-Ille-Pro at the C-terminus; CA-074, and also cath. L specific inhibitors; CLIK-148. (b) As the aliphatic aldehyde derivatives, cath.s specificity inhibitor, CLIK-60. (c) As the pyridoxal derivatives for cath-K, CLIK-164. These inhibitors showed specific inhibition for special cath.s at the 10\textsuperscript{-5} – 10\textsuperscript{-7} M level, in vitro and in vivo. 2) Medical applications. (a) Osteoporosis and bone metastasis of cancer were protected by cath-L or K inhibitor, CLIK-148 or CLIK-164. (b) Antigens are processed by various cath.s. T1-type and T2-type expressions were switched by antigen processing by different cath.s. (c) MHC Class II was activated by invariant chain degradation by cath.S. (d) In autoimmune Sjogren's disease, the auto-antigen "Hodrin" was processed by cath.S. CLIK-60 suppressed the Sjogren's syndromes in the model mice.

Conclusions: 1) Specific cath. inhibitor were designed and developed. 2) Osteoporosis and bone metastasis of cancer were suppressed by cath.L inhibitor. 3) Antigen processing and presentation were regulated by these inhibitors. 4) Suppression of autotagien processing in Sjogren D. by CLIK-60 and type-1 Diabetes by CLIK-148.

Melatonin: A Possible Magic Bullet in Reducing Hypoxic Brain Injury

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Melatonin, a neurohormone synthesized and secreted by the pineal gland is reported to have antioxidant, immunoregulatory and neuroprotective actions. Production of melatonin is regulated by light and darkness, light decreasing and darkness increasing its life. The production is also known to decline in old age and under hypoxic-ischemic conditions. Melatonin is considered the body's chronological pacemaker and has a wide array of useful applications. It has been used in the treatment of sleep disorders, especially those associated with circadian dysrhythmia, and is also reported to have neuroprotective effects in many central nervous system (CNS) conditions such as amyotrophic lateral sclerosis, Parkinson’s disease, Alzheimer's disease, ischemic injury, neuropsychiatric disorders and head injury. Results from our laboratory have shown that it affords protection to the blood- brain and blood-retinal barriers in hypoxic conditions by suppressing the production of vascular endothelial growth factor and nitric oxide which are known to increase vascular permeability. Protective effects of melatonin against hypoxic damage have also been demonstrated in newborn experimental animals where it suppressed damage in many parts of the brain such as the hippocampus and choroid plexus in lateral ventricles. Along with this, exogenous administration of melatonin in newborn animals has been shown to be effective in enhancing the surface receptors and antigens on the macrophages/microglia in the CNS supporting its immunoregulatory actions. Keeping these beneficial effects in view, melatonin merits consideration as a potential "magic bullet" for mitigating brain damage in hypoxic-ischemic injuries.

This study was supported by a research grant (R-181-000-098-112) from the National University of Singapore.

Cardiac Side Effects Of Psychotropic (Antidepressant, Antipsychotic) Drugs

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Background: The most frequent cardiac side effects of psychotropic drugs (antidepressants, antipsychotics) are bradycardia or tachycardia, ECG alterations (prolongation of QRS, QT interval), AV-block, ventricular arrhythmias (tachycardia, torsades de pointes, TdP) and sudden death.

Aims and methods: To attempt to find relations between clinical data and the electrophysiological effects of antidepressants (fluoxetine, citalopram), antipsychotics (risperidone) obtained in isolated guinea-pig ventricular muscles and canine ventricular myocytes using the conventional microelectrode and whole cell clamp technique.

Results: Fluoxetine (F) (0.5-50 µM) and citalopram (C) (10-100 µM) exhibited depressant effects on contraction and both on Ca\textsuperscript{2+} and Na\textsuperscript{+} dependent electrophysiological parameters of cardiac preparations and on cardiac Ca\textsuperscript{2+} current, without modifying the K\textsuperscript{+} currents. Risperidone (R) (0.1-10 µM) caused a concentration-dependent lengthening of action potential duration (APD) in both preparations and it blocked concentration-dependently the rapid component of the delayed rectifier K\textsuperscript{+} current (I\textsubscript{K1}). The other K\textsuperscript{+} currents (I\textsubscript{Kah} and I\textsubscript{Kur}) and Na\textsuperscript{+} current were not significantly modified. Conclusion: The inhibition of cardiac Ca\textsuperscript{2+} and Na\textsuperscript{+} currents by F and C, moreover the depression of I\textsubscript{K1} current by R may explain the cardiac side-effects observed occasionally with these drugs. Our results suggest that the new generation of antidepressants (fluoxetine, citalopram) and antipsychotics (risperidone) may have also antiarrhythmic, as well as proarrhythmic properties. Therefore, clinicians should be more vigilant about these potential adverse reactions and ECG control may be suggested during therapy, especially in patients with cardiovascular disorders.
Background: Fluoroquinolones have become the mainstay of treatment for typhoid fever in many countries, including South Africa. Resistance to the quinolones antibiotics results in treatment failures and quinolone resistance in South African strains of Salmonella enterica serotype Typhi is well documented.

Methods: The molecular mechanism for quinolone resistance in Salmonella Typhi from South African clinical isolates received by the Enteric Diseases Reference Unit of the National Institute for Communicable Diseases for the period 2003-2005 was determined using pulsed-field gel electrophoresis (PFGE), multiple-locus variable-number tandem-repeats analysis (MLVA), PCR and sequencing of the quinolone resistant determining region (QRDR) genes gyrA, gyrB, parC and parE; as well as plasmid-mediated quinolone resistance determinants (PMQR) qnrA, qnrB, and qnrS.

Results: PFGE showed 2 major clusters (90%) among the 20 quinolone resistance isolates. MLVA was more sensitive and grouped these same 20 isolates into 11 MLVA types, with the majority (8/20) grouped as MLVA type-16 with the other types differing by 1 allele, either TR1 or TR2. Among the 8 related and non-related PFGE isolates screened for mutations in the QRDR region 2/8 isolates had mutations in gyrA, parC and parE; 2/8 isolates had mutations in parE; 1/8 isolate had a mutation in gyrA and parE; 1/8 isolate had mutations in gyrA, gyrB, and parE; and 2/8 isolates exhibited no mutations in their QRDR regions. PCR screening for PMQR and qnrS were all negative.

Conclusions: Although these results seem counter-intuitive to previously published work, the molecular mechanism of quinolone resistance for these isolates may not be attributed to a single mechanism but may be the result of a combination of mechanisms.

Designing Drugs for Neurological Disorders: TRH-based Neurotherapeutics

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The trend in neurotherapeutic drug design is moving from a 'magic bullet' to a 'magic shotgun' approach, yet the naturally occurring neuroactive peptide thyrotropin-releasing hormone (TRH) has potential to act as a 'magic bullet' in the treatment of a wide variety of CNS disorders by virtue of its multifaceted homeostatic neurobiological actions. Clinical use of TRH is restricted, however, because of its short half-life due to degradation by TRH-degrading ectoenzyme (TRH-DE). We report the development of novel compounds that offer multifaceted homeostatic neurobiological actions. Clinical use of TRH is restricted, however, because of its short half-life due to degradation by TRH-degrading ectoenzyme (TRH-DE).

The world, beleaguered by a multitude of illnesses and syndromes, anxiously looks to pharmaceutical companies for the creation and distribution of substances that will ease pain, discomfort, and even reverse degeneration. However, a glance through pharmaceutical history tells us that a drug can be both a welcomed panacea and a despised scourge, a literal 'magic bullet.' Methamphetamine (meth) is one such substance. A derivative of amphetamine, which was first synthesized in 1887, methamphetamine has roots in 1940s Japan. It mirrors the 'magic bullet' to a 'magic shotgun' approach, yet the naturally occurring neuroactive peptide thyrotropin-releasing hormone (TRH) has potential to act as a 'magic bullet' in the treatment of a wide variety of CNS disorders by virtue of its multifaceted homeostatic neurobiological actions. Clinical use of TRH is restricted, however, because of its short half-life due to degradation by TRH-degrading ectoenzyme (TRH-DE). We report the development of novel compounds that offer multifaceted homeostatic neurobiological actions. Clinical use of TRH is restricted, however, because of its short half-life due to degradation by TRH-degrading ectoenzyme (TRH-DE).

Results/Conclusions: The widespread use of HAART has reduced the incidence and complications of CMV retinitis in patients with HIV infection. With sustained immune recovery, discontinuation of anti-CMV therapy has been possible in many patients. Still, immune recovery does not guarantee protection from recurent disease. CMV retinitis and uveitis associated with immune recovery remain causes of vision loss in this population. Areas such as genetic susceptibility to CMV retinitis and the development of long-term drugs and drug delivery vehicles appropriate for developing countries offer further avenues of investigation.

Background: Cytomegalovirus (CMV) is a common opportunistic infection in individuals with AIDS, with CMV retinitis representing a significant portion of end-organ disease in these patients. Before the development of highly active antiretroviral therapy (HAART), nearly one-third of people with AIDS developed CMV retinitis during their lifetime. Although effective therapies for CMV infection had been developed, treatment was often life-long due to persistent immune deficiency. Even with chronic suppressive maintenance therapy, disease relapse was nearly universal, and development of drug resistance was not uncommon. Aims: 1) To evaluate the impact of HAART on the course and complications of CMV retinitis 2) To identify continued challenges in the treatment of CMV retinitis and propose further avenues for investigation.

Methods: This study reviewed available evidence in the medical literature concerning the treatments and outcomes of CMV retinitis with particular attention to the impact of HAART and to data derived from the Longitudinal Study of the Ocular Complications of AIDS.

Results/Conclusions: The widespread use of HAART has reduced the incidence and complications of CMV retinitis in patients with HIV infection. With sustained immune recovery, discontinuation of anti-CMV therapy has been possible in many patients. Still, immune recovery does not guarantee protection from recurent disease. CMV retinitis and uveitis associated with immune recovery remain causes of vision loss in this population. Areas such as genetic susceptibility to CMV retinitis and the development of long-term drugs and drug delivery vehicles appropriate for developing countries offer further avenues of investigation.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
ODAM As A Diagnostic And Therapeutic Target For Human Breast Cancer
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Background: We have previously reported that the structurally novel Osteodentritic Ameboblaster Associated Protein (ODAM), expressed in ameboblasts during late tooth development and in osteodentritic tumors is also found in human breast cancer (Kestler et al. 2008 Mol Med 14:318-323).

Methods: To investigate the possible role of ODAM as a biomarker of breast cancer, as well as a potential diagnostic and therapeutic target, we have tested the capabilities of our anti-ODAM mAbs to immunostain a) mouse mammary tissue from different stages of development; b) human breast cancer arrays, and c) biopsy specimens obtained from patients with stages I-IV breast cancer. Additionally, we have used dual micro-SPECT/CT to image mouse mammary tumor xenografts with an 125I-labelled anti-ODAM mAb.

Results: Tissues from all stages of mouse mammary development except lactation expressed ODAM; further, a significant number of specimens contained in human breast carcinoma arrays also were stained by these reagents. Among 60 patient samples analyzed in a retrospective study, ODAM expression was significantly greater in advanced (stage IV) than in early (stages I-III) disease. The 125I-anti-ODAM mAb was also capable of imaging a murine mammary xenograft. Furthermore, we found (using an ELISA-based procedure) that the sera of patients with metastatic breast cancer contained elevated titers of anti-ODAM antibodies.

Conclusions: Our finding that ODAM expression in human breast cancer correlates with disease stage has prognostic import. The presence of anti-ODAM antibodies in the sera of patients with metastatic disease also is of note, given that autoantibodies to growth regulatory factors have been detected in individuals with other types of malignancies in which the titers of these components have correlated with survival and other clinicopathological parameters. Additionally, radiolabeled anti-ODAM mAbs may prove useful to document the presence of tumor metastasis or relapse. Based on our data, we posit that ODAM has a functional role in breast development and in the pathogenesis of breast cancer where it could serve as a novel diagnostic and therapeutic target.

ODAM in the Postoperative Small Intestine
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Background: The effects of surgery on gastric emptying have been documented for a considerable time, but less is known about the effects in the small intestine. It is thought that there is minimal diminution in the absorptive capacity of the small intestine postoperatively, although there is no literature on drug absorption in the early period after surgery. This study investigated drug absorption from the small bowel in patients undergoing abdominal surgery.

Methods: A prospective study of patients undergoing major abdominal surgery in which patients acted as their own pre-operative controls was carried out. Patients were administered the test drugs, paracetamol and 111In-DTPA-py, pre-surgery and two days postoperatively. Small intestine transit times, plasma concentrations and other pharmacokinetic variables were compared using Student’s paired t test.

Two complementary studies were carried out to establish pharmacokinetic parameters.

Results: There were no significant differences in the pre- and postoperative values of tmax, AUC, and AUMC pre- and postoperatively, (p > 0.05). There were significant differences between the pre- and postoperative values of tmax (p > 0.05) and the pre- and postoperative values of MRT (MRT (preop) < MRT (postop); p < 0.01).

Conclusions: Drug absorption from the small bowel in the postoperative patient does not differ significantly from its preoperative absorptive capacity.

Treatment Prospects for Breast Cancer: Lessons Learnt from a Decade of Research on Maspin
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Background: Maspin is a multifaceted protein, interacting with a diverse group of intra- and extra-cellular proteins, regulating cell adhesion, motility, apoptosis, angiogenesis and critically involved in mammary gland development. Maspin is a cytosolic protein but is also localized to the nucleus and membrane, and is secreted. Aberrant methylation of the Maspin promoter is closely associated with Maspin gene silencing and is a common occurrence in cancer. Our laboratory has identified the aspartyl endopeptidase Cathepsin D (CatD) as a binding partner for Maspin, which is downregulated in primary tumors and absent in metastatic breast cancer. CatD is excessively produced and aberrantly secreted by tumor cells. Studies depict a critical role for CatD in tumor growth (mitogenic effect), and invasion (proteolytic effect on matrix components) in breast cancer. Based on the importance of both Maspin and CatD, and specifically the reciprocity of their relationship in breast cancer, we embarked on identifying factors which might influence the Maspin and CatD partnership under normal conditions. Such an approach would shed light on how their alteration could lead to malignant growth and ultimately metastasis.

Methods: We employed in vivo (mice model of mammary gland development), and in vitro (normal mammary epithelial and breast cancer cell lines grown on 3D matrixes) models to decipher the Maspin and CatD partnership in the context of mammary gland development and during neoplastic breast cancer progression.

Results: Our studies have illuminated a previously unidentified function for Maspin and its interaction with CatD in maintaining the differentiated secretory glandular phenotype of the mammary gland. In addition, the secretion of Maspin by mammary epithelial cells and its deposition into the extracellular milieu plays an important role in matrix degradation by CatD. In this capacity Maspin could potentially regulate mammary tissue remodeling occurring under normal and pathological conditions.

Conclusions: Studying this unique partnership has provided us with a critical view into several previously unidentified mechanisms of action for both of these proteins, and may contribute new strategies underlying Maspin- and CatD-based therapeutic approaches for combating breast cancer.
Anti-inflammatory, analgesic and antipyretic activities of Physalis minima Linn

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Background: To evaluate anti-inflammatory, analgesic and antipyretic activities of Physalis minima Linn in order to discover natural remedies for the management of various painful and inflammatory conditions.

Methods: MNN mice (22–28 g) and Wistar rats (180–200 g) of either sex were obtained from the animal house facility of H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan. The carrageenan induced hind paw edema and Cotton pellet-induced granuloma tests were conducted for both crude extract and chloroform fraction. While the Acetic acid-induced abdominal constriction and Formalin test were performed to evaluate the analgesic potential of crude extract and chloroform fraction. The crude extract and chloroform fraction was also tested against Brewer’s yeast (Saccharomyces cerevisiae), induced fever.

Results: The crude extract (58%) and chloroform fraction (62%) of Physalis minima significantly inhibited the carrageenan induced paw edema in rats at 400 mg/kg. In a dose dependent manner at 400 mg/kg, the crude methanol extract and chloroform extract fraction reduced granuloma (48%) and (62%) respectively. In a dose dependent manner at 400 mg/kg, the crude extract and chloroform fraction reduced the number of abdominal constriction (52%) and (38%) respectively. The crude methanol extract (51%) and chloroform fraction (31%) activity in dose dependent way in the late phase in formalin induced pain. In case of antipyretic assay, the crude extract and chloroform fraction of Physalis minima exhibited insignificant activity. Values of p < 0.05 were considered significant in all cases.

Conclusions: Both the crude extract and chloroform fraction of the plant showed significant anti-inflammatory and anti-inflammatory activity as compared to control, while the anti-pyretic response was insignificant.

New Steroidal Hormones Promise to Become a Multi-Purpose “Magic Bullet”

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Background: Discovery of plant steroid hormones called brassinosteroids (BS) showed that steroids are versatile hormonal regulators, characteristic to most organisms. To the Ehrlich, BS demonstrate wide regulatory effects on plant growth and development. Their important feature is the ability to enhance plant resistance to unfavorable factors (diseases, stresses, pollutants, etc.) and to improve plant productivity together with the quality of crops. BS protective action is a result of multiple changes at molecular and cellular levels including activation of protein and nucleic acids’ biosynthesis, changes in hormonal balance, activity of enzymes, in composition and properties of membranes, in activity of different systems of plants. BS have been and are consumed by mammals with food over all the evolution, but till recently there were looking at BS from a new point of view: they are promising multi-purpose agents for human and veterinary medicine.

Methods: The work is devoted to the study of the in vitro and some other data reflecting its action on immune and hormonal system of animals. Effects on the serum cholesterol levels were studied in rats and human volunteers. Anti-HIV activity was studied using Formazan assay, Supravital cell staining by the trypan blue assay and Indirect immunofluorescence assay. Some effects of EBl on immune, hormonal and reproductive systems were investigated in mice, in rats and in chickens and in fishes.

Results: The study showed a high efficiency of EBl as a cholesterol-lowering agent in mammals for a wide range of doses. Tests on anti-HIV activity showed that EBl is efficient as anti-viral agent at average concentration of 10 μM. Effect on hormonal balance and stimulation of immune and reproductive system were registered.

Conclusions: The obtained data provide evidence that BS possess different activities in animals, and these activities are similar to a certain extent to those we know in plants both in respect to profile and active doses. This finding allows looking at BS from a new point of view: they are promising multi-purpose agents for human and veterinary medicine.

Peptides Against Ageing

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Background: Ageing is characterized by desorganization of peptidic system of regulation of organism functions. The development and study of peptide geroprotectors is very prospective.

Methods: The level of expression of various genes, intensity of protein synthesis in the cells, indices of immune and endocrine system, metabolism and antioxidant defense were studied in animals and humans.

Results: Peptides regulated gene expression and protein synthesis in the cells. This was largely conditioned by immunomodulating, oncomodifying and stress-protection properties of peptides. Peptides contribute to DNA structure restoration and decreased incidence of chromosome aberrations in the cells induced by radiation, chemical effects and hypoxenia. Mechanisms of their geroprotective action are related to activation of chroinat in blood lymphocytes of old patients. Peptides show opioid activity and produce modulating effect on the content of biogenic amines (noradrenaline, dopamine, 5-oxoiodoacetic acid, serotonin, histamine) in brain cortex and blood serum of animals, due to their effect on the central and peripheral regulatory mechanisms of stress and inflammation. Geroprotective action of peptides is related to their influence on the mechanisms of hormone regulation and antioxidant defense. Administration of thymus and pineal peptides to mice and rats of different strains promoted reliable increase in an average life span by 30-40% and depressed growth of spontaneous, induced and transplanted tumors in animals. Animals revealed restoration of the regulatory function of the hypothalamic-pituitary-gonadal axis in several cases of spontaneous, induced and transplanted tumors in animals. Animals revealed restoration of the regulatory function of the hypothalamic-pituitary-gonadal axis in several cases. Administration of pineal peptides to old monkeys promoted reliable restoration of the hypothalamic, gastrointestinal and cardiovascular systems, brain functions. It was accompanied by a 2-fold decrease in the mortality rate in these patients during 8-12 randomized clinical trials.

Conclusions: The results of studies evidence prospects for application of peptide geroprotectors for prevention of premature ageing, age-related pathology and an increase in the period of active longevity.

From 1-4 Weeks Of Treatment Down To A Single Application: A Novel Terbinafine Topical Treatment Of Tinea Pedis

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Background: Tinea pedis is a common dermatophytosis requiring topical antifungals for at least 1-4 weeks (W). This has a negative impact on compliance and outcomes. A novel topical solution of terbinafine (film-forming-solution - FFS) was developed to allow single application.

Methods: 1- The stratum corneum (SC) pharmacokinetics (PK) of terbinafine following single application was investigated in three PK trials on healthy volunteers (n = 6, 12 and 18). Drugs were applied to the back and skin strips were taken from defined areas at baseline and from 1 to 312 h after application. Samples were analysed using validated liquid chromatography/mass spectrometry. 2- Dose-finding trials: 10%, 5%, 1% and 0.5% FFS were non-inferior to 1% FFS in some components of mitochondria respiratory chain. Administration of pineal peptides to old monkeys promoted reliable restoration of the hypothalamic, gastrointestinal and cardiovascular systems, brain functions. It was accompanied by a 2-fold decrease in the mortality rate in these patients during 8-12 randomized clinical trials.

Results: 1- The residence time of the film on the skin was up to 72 h, 30% of the total amount of drug delivered into the SC occurred during the first 2 h, 31% from 2-12 h, and 39% thereafter. The maximum concentration was observed as early as 1.5 h. Pseudocidal SC terbinafine levels were still detected after 13 days (24 ng/ml). 2- ETR at W 6 with 10%, 5% and 1% FFS were 61%, 70%, 66% compared to 18% with the vehicle. All three active treatments were significantly superior to the vehicle (P<0.001). 1% and 5% FFS were non-inferior to 1% FFS. In the efficacy trial, ETR was 63% in the 1% FFS group and 17% for the vehicle (P<0.001). Recurrence occurred in 12.5% of the effectively treated patients at W 12 was also assessed.

Results: 1- The residence time of the film on the skin was up to 72 h, 30% of the total amount of drug delivered into the SC occurred during the first 2 h, 31% from 2-12 h, and 39% thereafter. The maximum concentration was observed as early as 1.5 h. Pseudocidal SC terbinafine levels were still detected after 13 days (24 ng/ml). 2- ETR at W 6 with 10%, 5% and 1% FFS were 61%, 70%, 66% compared to 18% with the vehicle. All three active treatments were significantly superior to the vehicle (P<0.001). 1% and 5% FFS were non-inferior to 1% FFS. In the efficacy trial, ETR was 63% in the 1% FFS group and 17% for the vehicle (P<0.001). Recurrence occurred in 12.5% of the effectively treated patients at W 12 was also assessed.

Conclusions: This novel formulation delivers high amounts of terbinafine to the SC for a prolonged time. 1% FFS was the minimal effective dose. Effectiveness of 1% FFS was confirmed by the efficacy trial which also showed a similar relapse/re-infection rate to that previously demonstrated with terbinafine 1 % SC for a single 2-week application. This novel product represents a significant advance in the treatment of tinea pedis with the enhanced compliance and convenience that it offers.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
The morbidity and mortality from asthma in the Western world have profoundly increased in the past two decades. Recent studies showed that sensitization and exposure to cockroach allergens strongly correlated with increased asthma morbidity and severity for children, especially among inner city children. As a unique form of chronic airways inflammation, asthma is characterized by reversible airway obstruction, airway hyperresponsiveness, and the production of multiple inflammatory mediators. Local activation of both immune and nonimmune cells in the lung triggers the release of these immunomodulator proteins including tumor necrosis factor (TNF) and TNF-α, as a multifunction pro-inflammatory cytokine, has been postulated to be a critical mediator directly contributing to the bronchoconstrictor inflammation and airway hyper-reactivity in asthma. The successful treatment of various chronic inflammatory diseases such as rheumatoid arthritis, Crohn’s disease, and psoriasis provides great potential that inhibition of TNF-α activity may have application for the treatment of asthma. Recently we have shown that airway expression of TNF-α peaked shortly after allergen challenge in a mouse model of asthma induced by a house dust extract that contains high level of cockroach allergen and endotoxin. TNF neutralization with a specific antibody significantly reduce the pulmonary inflammation and airway hyperresponsiveness. Recent developments in clinical trials in patients with severe asthma provide strong support for the concept that blocking TNF-α activity represents a new approach in asthma therapy.

Quinolone Resistance in Campylobacter isolates Originating From Chicken in Senegal.

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Methods: Multilocus sequence typing (MLST) was used to study the clonality of isolates.

Results: In Senegal, fluoroquinolones (norfloxacin and enrofloxacin) were first used in poultry production in 1996 to treat respiratory and intestinal diseases.

Conclusions: C. jejuni and C. coli strains isolated from chicken in Senegal.

Opioid Antagonist or Opioid Antagonist: Magic Bullets in the Treatment of Opioid Addiction

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Background: Methadone maintenance treatment for chronic opioid addiction was envisioned as a “magic bullet” to eliminate opioid withdrawal and craving to make meaningful rehabilitation possible. Methadone maintenance over long periods was undesirable to some patients, yet discontinuation of methadone maintenance often led to heroin relapse even after long periods of abstinence. An alternate oral active “magic bullet”, naltrexone, was developed to improve overall acceptability of opioid addiction treatment. It blocks the opioid receptor and prevents opioid physical dependence. Unfortunately, this highly efficacious treatment is poorly effective because patient adherence is worse than that for methadone maintenance. The effectiveness of both medications is significantly limited by patient non-adherence, so systems of care must be developed to ensure delivery of the medication and other services needed for optimal rehabilitation. This “magic gun” for the “magic bullet” is key to the treatment process, but is an element often lacking in treatment of chronic medical problems.

Methods: We developed Motivated Stepped Care (MSC) as a “magic gun” to improve the effectiveness of methadone maintenance by using the behaviorally reinforcing properties of methadone to motivate improved adherence to a stepped care, patient-treatment matching paradigm. Patients enter MSC in low intensity counseling care and are referred to higher, discrete intensities (“steps”) based on objective indicators of current treatment response. Once stabilized, they are returned to lower steps of care in an efficient and cost effective manner. A randomized, controlled trial of 127 new admissions was used to evaluate the effectiveness of MSC.

Results: Patients randomly assigned to MSC (n=65) had lower rates of poor treatment response (46% vs 79%, p < .001), and improved counseling attendance (83% vs 44%, p < .001) compared to a standard treatment condition (n=62). MSC was well tolerated and associated with excellent attendance across varying treatment schedule intensities.

Conclusions: 1) Treatment adherence and response to MSC is superior to standard methadone maintenance treatment. 2) This treatment approach has broad theoretical and practical application in treatment of addiction and other chronic behavioral problems that share the common problem of poor treatment adherence.

Effect Of A Subtoxic Dose Of Acetaminophen On The Toxicity Of Chemicals That Are Metabolically Activated

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Background: While numerous studies describe the toxic consequences resulting from an excessively large dose of acetaminophen (APAP), a widely used analgesic-antipyretic, its potential adverse effects at a lower or therapeutic dose have hardly been explored. The present study was aimed to examine the effects of prior exposure to a subtoxic dose of APAP on the metabolic disposition and toxicity of a following dose of this drug.

Methods: In a preliminary experiment an APAP dose of 500 mg/kg, ip, was shown to be non-toxic to female Sprague Dawley rats used in this study. At 18 h after administration of APAP at this dose, rats were challenged with an identical dose of APAP and the elimination of APAP from blood was determined. Serum enzyme activities were measured 24 h after the challenging dose of APAP to estimate the liver injury. Also the hepatic microsomal drug metabolizing enzyme activities and their expression were measured in rats treated with a single dose of APAP 18 h prior to sacrifice.

Results: APAP and APAP-glucuronide concentrations in plasma were unaltered by APAP pretreatment. APAP-sulfate concentrations were decreased, while APAP-nitrosoconjugates concentrations were elevated significantly. The elevation of serum hepatotoxic parameters was also enhanced by APAP pretreatment. In rats treated with a single dose of APAP 18 h prior to sacrifice, hepatic microsomal chlorozoxazine 6-hydroxylase, p-nitrophenol hydroxylase, p-nitroanisole O-deethylase, and acetylation N-demethylase activities were all increased to 173 %, 151 %, 158 %, and 116 % of normal control, respectively. Immunoblot analysis showed that expression of CYP2E1, 3A, and 1A was also increased significantly. Neither hepatic glutathione contents nor glutathione S-transferase activity was changed by the single dose of APAP.

Conclusions: 1) A subtoxic dose of APAP may increase the CYP2E1, 3A, and 1A expression and their metabolizing activities. 2) The altered CYP contents and activities may actually influence the metabolism and resulting toxicity of a repeated dose of APAP. 3) Considering the wide use of APAP as an analgesic-antipyretic, it is suggested that a greater concern should be expressed regarding the effects of acute or repeated dosing of this drug even at a therapeutic level, especially when used in combination with other medications.
Exploiting Plant Sources for Potential Drugs. Alpinumisoflavones in Perspective

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Background: Alpinumisoflavones have been known to have a variety of medicinal properties and currently several medicinal in aphrodisiac medical practice in Sub-Saharan countries and different cultures. Scientific investigation has established a myriad of biological in vivo studies purporting their unique use in folklore medicine several of which have been catalogued. In French West Africa, the task is not as easy as in countries of Nigeria, in the northern part of the task is not easy but do exist. Studies and trials in folk medicine are the most reliable and acceptable in the world, but also the most difficult to perform and interpret. Although, the process of development and purification of these compounds is not yet well exemplified. (Maquet et al., 1990; Meadell et al., 1996), a wild mainly commercializing the microorganisms that cause cholera, schistosomiasis, Trypanosomiasis, a venereal disease endemic throughout South Africa, Africa and the Far East. Our recent investigation into the structure and biotransformation and clinical outcome. In antidepressant drug treatment, genetic polymorphisms in drug metabolizing enzymes as well influence pharmacokinetic parameters to a large extent. Recent studies have shown a relationship between polymorphic drug effects and CYP2D6 genotype. A prospective evaluation of the cost-benefit of genotyping in this field would be very helpful for the aim of introducing pharmacogenetic diagnostic into drug therapy.

Anti-Cardiovascular Specific Immune Response And Tumor Cell Lysis

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Background: Tumor-associated antigens resulting from aberrant glycosylation, such as the SialylTn antigen, are frequently expressed on cancer cells and provide potential targets for vaccination. Carbohydrates, however, being T-cell independent antigens, are poorly immunogenic and fail to induce a memory response. To increase immunogenicity, SialylTn antigen was coupled to a highly immunogenic carrier, the murine monodonal antibody mAb17-1A. An immunomagnetic fusion of SialylTn-mAb17-1A conjugate on aldehyde (designated MB 402) with or w/o additional adjuvants was tested in Rhesus monkeys for tolerability and immunogenicity.

Methods: The SialylTn antigen was coupled to the mAb17-1A (mitg22a) carrier. The coupling product was analyzed by SEC-HPLC, LDI-PAGE, Western blot, and IEF analysis. A suspension of murine monoclonal antibodies (mAb17-1A) was treated with trichloroacetic acid and the samples were analyzed by immunoblotting. Safety, tolerability and immunogenicity of multiple injections of MB 402 were evaluated in Rhesus monkeys vaccinated four times by s.c. injection and boosted on day 226. Blood samples were taken before and after immunization for serum analysis.

Results: Immunogenic response against mAb17-1A, SialylTn and Ovine submaxillary mucin, and SialylTn+ (tumor cells) were measured. Cytokine release in serum was analyzed using xMAP Multiplex technology. NK lysis of tumor cells was measured using a 3H-release assay.

Conclusions: The present study demonstrates that synthetic vaccines eliciting specific immune response against defined target antigen(s) together with a synchronized cytokine release are promising candidates for cancer vaccines.

Pharmacogenetic diagnostics for optimization of psychotropic drug treatment

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For many drugs, pharmacogenetic polymorphisms are known affecting bioavailability and clinical outcome. The clinical relevance of these variations depends on allele-frequency and the effect size of the clinical outcome parameter. Pharmacogenetic polymorphisms range in the range of the drug which is affected, on predictability of drug response as well as on duration until onset of therapeutic efficacy. Consequences which arise from genotyping might be: adjustment of drug therapy, to genotype, choice of therapeutic strategy or even choice of drug.

For many psychotropic drugs, pharmacogenetic polymorphisms are known affecting bioavailability and clinical outcome. In antidepressant treatment, most drugs are metabolized via the polymorphic cytochrome P450 enzymes CYP2D6 and CYP2C19. Huge differences in pharmacokinetic parameters have been consistently shown for many tricyclics, some SSRIs, and other antidepressant drugs. However, the effects on therapeutic efficacy and adverse events have been described controversially. Pharmacogenetic polymorphisms can be overcome by adapting the drug dosages and dosing intervals. Similar to bioequivalence studies, the aim to achieve similar plasma concentration time courses of antidepressants might help to reduce side effects and therapeutic failure.

In the field of antidepressant drug treatment, genetic polymorphisms in drug metabolism as well influence pharmacokinetic parameters to a large extent. These kinds of drug therapy, a more clear dose dependency of side effects such as extrapyramidal side effects exists, and the combination of genetic polymorphisms might be more beneficial. Recent studies showed a relationship between the occurrence of adverse side effects and CYP2D6 genotype. A prospective evaluation of the cost-benefit of genotyping in this field would be very helpful for the aim of introducing pharmacogenetic diagnostic into drug therapy.

Organ Independent Drug Elimination

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Background: Citracurium is a bisquinoindolimine nondepolarizing neuromuscular blocking agent. It has been used for skeletal muscle relaxation during surgical procedures and during mechanical ventilator support. It has been shown that the major route of citracurium clearance is through the organ independent process of Hofmann elimination (pH and temperature dependent). Hofmann elimination takes place ubiquitously in plasma and tissue, and accounts for approximately 5% of total clearance of citracurium. Tight physiologic control of pH and temperature, maintaining Hofmann elimination, results in low interpatient variability in the CL of citracurium (161). The aim of the current study was to examine the relationships between pharmacokinetic parameters in the setting of organ independent elimination.

Methods: Geometric regression analysis was performed to look at the relationships between clearance and half-life, volume of distribution and half-life, and clearance and volume of distribution in 31 otherwise healthy patients who underwent minor surgical procedures requiring tracheal intubation.

Results: For citracurium, geometric regression analysis revealed that there is no relationship between clearance and half-life (r = 0.91; p < 0.001) nor is there a relationship between citracurium volume of distribution and half-life (r = 0.31; r = 0.04; p < 0.001).

Conclusions: Citracurium was being "removed" from the body mainly by the organ independent route of Hofmann elimination results in unique relationships among pharmacokinetic parameters. Conventional relationships between pharmacokinetic parameters, with the primary parameters of CL and Vd, being independent and the secondary parameter of t1/2 being dependent on CL and Vd, do not hold for citracurium as the half-life is essentially "fixed" by Hofmann elimination. Half-life of antidepressive drugs and Vd, while the CL and Vd are directly related. Other compounds that undergo organ independent elimination via a ubiquitous mechanism may also display these unique characteristics with respect to pharmacokinetic parameter relationships.

Genetic And Epigenetic Effects Of Pharmacological Doses Of Gamma-Hydroxybutyrate (GHB) In The Rat Brain

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Background: Gamma-hydroxybutyrate (GHB) is at the same time an endogenous neuromodulator of some brain synapses at micromolar concentrations, a therapeutic substance used to induce deep sleep in narcoleptic patients or for anesthetic purposes, an agent that has been proposed to alleviate withdrawal symptoms in alcoholics and also a substance of abuse which can induce addiction. In order to obtain therapeutic or recreational effects, large doses of GHB must be administered or absorbed. The principal targets of these pharmacological doses of GHB are primarily the brain endogenous GABAergic system and the GABAAergic system. However, it can be predicted that millimolar brain concentrations of GHB will target different proteins and finally adapt the expression of several genes.

Methods: The present study focuses on the transcriptome modifications and HDAC inhibition due to GHB overload in two brain regions, the hippocampus and frontal cortex, where some genes are also similarly affected in both structures. As it become evident that important epigenetic mechanisms are at the basis of modifications in gene expression and regulate drug addiction, we demonstrate that acute pharmacological doses of GHB increase histone H3 acetylation in both brain structures. These results were confirmed by quantitative immunocytochemical studies and by in vitro HDAC inhibition by GHB.

Results: If we exclude the large proportion of modified EST (54.9% of all probes set), numerous functional important genes were differentially regulated. These concern first neuronal signaling and metabolic processing, then regulation of DNA transcription, stress response and neuronal growth or structure. Several differences exist between hippocampus and frontal cortex, but some genes are also similarly affected in both structures. As it becomes evident that important epigenetic mechanisms are at the basis of modifications in gene expression and regulate drug addiction, we demonstrate that acute pharmacological doses of GHB increase histone H3 acetylation in both brain structures. These results were confirmed by quantitative immunocytochemical studies and by in vitro HDAC inhibition by GHB.

Conclusions: The present study completes the classical model which described pharmacological effects of GHB and shows that acute doses of this substance exerts a profound effect at the level of gene transcription, probably partly due to modifications of the accessibility of chromatin to regulatory factors.

Background:

Human serum albumin (HSA, fatty acid free), and fluorine-containing ultraviolet spectra of the drugs in HSA buffer solutions (pH 7.40) were measured to calculate the binding constants (Ks). Structural study of the drug binding and the effects of the endogenous substances on it were performed by 19F NMR spectrometry.

Results:

A single sharp 19F NMR signal of each drug in buffer solutions was split and broadened by addition of albumin, revealing that the drug bind at more than one site. From competitive 19F NMR experiments using known ligands, TFZ and TFZP were found to bind to site I and another unknown site, and BFZ was to bind to both site I and II. Some of the 19F NMR signals of the bound drugs showed intensity reduction or increase upon addition of the endogenous substances. The K-values depending on the amount of oleate up to 0.8% of oleate increased the K-values depending on the amount of oleate up to three times of that of albumin, however, further addition reduced them. Ca-induced concentration-dependent suppression effect on the drug binding (TFZ, BFZ).

Conclusions:

1) Buffers to be used in the determination of the drug-albumin binding constants should contain 0.1 M Ca2+, otherwise, the binding constants may be over estimated. 2) The results of oleate suggested that the unbound free drug concentration may very depending on the amount of LCFA in the blood, which depends on physical conditions of the body. 3) Possible concentration fluctuation of Ca2+ in the blood may affect the free drug concentration.

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Discovery Of Dual-Targetting Ligands

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Background: A wealth of examples, both in literature and in the clinic currently exist whereby drugs have been shown to act via multiple targets, albeit these discoveries were generally retrospective. The intentional design of ligands that act at dual or multiple targets is becoming widely recognised as an approach to produce a single drug, in some cases with superior side-effect profiles and therapeutic effects, when compared with the combination of individual drugs. In general, methods such as generation of a conjugate ligand that incorporates functional groups key for binding to both targets are employed, with efficacy. However, this method often leads to the identification of higher MW compounds, as pharmacophores for both targets are not significantly overlapped.

Conclusions: In certain cases concentration of the drug in the skin should be determined instead of its plasma concentration. However, evaluation of skin concentrations cannot be replaced by their concentration in theoretical peripheral compartment.

The Pharmacology, Pharmacokinetics, Clinical Efficacy, Adverse Effects and Toxicities, Drug Interactions, Dosage And Administration, And Safety Issues Related To The Use Of Prasterone Are Discussed

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Prasterone is a proprietary synthetic dehydroepiandrosterone product under investigation for use in women with systemic lupus erythematosus (SLE) who are taking glucocorticoids. Initial trials investigated prasterone as a treatment to improve disease activity and quality of life in women with mild to moderate SLE. The Food and Drug Administration (FDA) did not approve prasterone's labeling for these indications. Subsequent trials have focused on prasterone as a treatment to limit bone loss in women who have SLE. A study was conducted to assess bone mineral density in patients who had been taking glucocorticoids for six months or longer. The patients in the prasterone group showed an increase in bone mineral density, while the placebo group demonstrated a loss. The most common adverse effects of prasterone therapy were acne and hirsutism. Hematia, hypertension, and serum creatinine concentration increases have also occurred. Interactions of prasterone potentially exist with 5-alpha reductase inhibitors and additive or antagonistic effects could possibly occur with androgens, estrogens, oral contraceptives, and progestins. In clinical trials, oral prasterone dosages of 150-200mg /day were administered. These dosages have resulted in supraphysiological hormone levels.

Conclusion: FDA has granted orphan drug status for the prevention of loss of bone mineral density in SLE patients taking glucocorticoids. FDA is requesting additional Phase III trial data for the treatment of SLE and the prevention of loss of bone mineral density.

Source: American Journal of Health-System Pharmacy. 63(22): 2201-10, 2006 Nov 15
Targeting Cell Cycle Progression by Troglitazone

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Background and Aim: Increasing evidence has confirmed that ligands for peroxisome proliferator-activated receptor γ (PPARγ) exhibit antitumoral effects through inhibition of cell proliferation and induction of cell differentiation in several malignant neoplasms. Recently, we have documented the accumulation of a cyclin-dependent kinase inhibitor, p27Kip1, as well as an unexpected accumulation in cyclin E in G1-arrested human hepatoma cells treated with the PPARγ ligand troglitazone. Simultaneous accumulations in both p27Kip1 and cyclin E are known to be characteristic phenotypes in cells derived from mice lacking Skp2, an F-box protein component of the SCF ubiquitin-ligase complex. Thus, the aim of the present study was to assess whether Skp2 might be involved in the up-regulation of p27Kip1 in troglitazone-treated human hepatoma cells.

Methods: Human hepatoma cell lines were used in this study. Cell cycle was analyzed by flow cytometry and immunoblotting. The mRNA levels of p21, p27, and cyclin E in the hepatoma cells were examined by quantitative real-time RT-PCR. Skp2-overexpressing hepatoma cells were generated by the cDNA transfection. The expression levels of Skp2 and p27Kip1 in human hepatocellular carcinoma (HCC) tissues and the adjacent noncancerous liver tissues were assessed by immunoblotting.

Results: A striking decrease in Skp2 expression and a reciprocal increase in p27Kip1 expression were found in troglitazone-treated hepatoma cells but not in those cells treated with other PPARγ ligands such as pioglitazone and ciglitazone. Quantitative real-time RT-PCR showed that troglitazone down-regulated Skp2 at the mRNA levels. Consistently, ectopic overexpression in Skp2-bound cells led to the formation of a decreased population of arrested cells at the G1 phase compared with that in the mock-transfected cells. In surgically resected hepatocellular carcinoma (HCC) tissue, an increased expression in Skp2 was found in both the moderately differentiated HCCs and the poorly differentiated HCCs.

Conclusions: Troglitazone attenuated Skp2 expression, thereby possibly promoting p27Kip1 accumulation in human hepatoma cells. This therapeutic potential of the ligand may lead to new cell-cycle-based antitumor strategies for advanced HCCs.

Enhanced Potency Of Antibodies Using Biologically Active Peptides

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Background: Antibodies have evolved as effective alternative to chemical drugs to treat cancers, yet clinical response is limited by expression of tumor targets reducing the potency of antibodies. We have invented methods to enhance potency by (1) generating polyvalent (homophilic) antibodies and by (2) making membrane penetrating antibodies. The objectives of these methods is to create antibodies with enhanced anti-tumor effects and for intra-cellular target-to-cell proliferation.

Methods: To demonstrate enhanced potency of homophilic antibodies the Her2/neu specific Herceptin was crosslinked with a homophilic 26mer peptide derived from a naturally occurring homophilic antibody, T15, previously discovered in our laboratory. The peptide was photo-actively affinity crosslinked to Herceptin. Homophilic Herceptin was compared to naked Herceptin in FACS, apoptosis induction and in xenograft animal model using the human lung tumor H1650. Transmembrane antibodies were generated by photo-affinity crosslinking a 14mer peptide derived from human sarkosi sarcoma virus. Intracellular targeting of life cells is demonstrated by confocal microscopy and inhibition of induced apoptosis.

Results: Homophilic Herceptin produced > 1 log stronger fluorescent intensity over naked Herceptin and increased the number of apoptotic cells to 82% compared to 12% with naked Herceptin. Homophilic Herceptin inhibited tumor growth by 60% in H1650 xenograft experiments compared to mice treated with naked Herceptin or in no-treatment controls.

Trans-membrane penetrating (TMP) antibodies stained in live cells specifically and in vivo while naked antibodies were not effective. TMP antibodies did not affect cell growth in culture. Furthermore, TMP modified anti-caspase3 antibodies blocked induction of apoptosis induced by actinomycin.

Conclusions: 1) Photo-affinity crosslinking of biologically active peptides endows antibodies with properties that enhance targeting and open up novel target opportunities. Homophilic peptide-modified Herceptin could show enhanced activity and improve current methods to treat tumors. 2) Membrane penetrating modified antibodies target intra-cellular antigens in live cells. Collectively, these data show a new approach in the creation of a novel class of antibodies that are superior as diagnostic tools and therapeutic drugs.
Nitric Oxide and Zidovudine Potentiate Oxidative Response of Stimulated Macrophages

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Background: Macrophage is one of the key cell types involved in many diseases. Therefore modulation of oxidative response (respiratory burst) of stimulated macrophages by various pharmacological agents is very important. The aim of this study was to test (separately) modulation of respiratory burst by zidovudine and nitric oxide in vitro.

Methods: Macrophages were isolated from the peritoneal cavity of 300-400 g male Sprague-Dawley rats 3 days following injection of casein. The cells were washed and incubated with 18 µM of nitric oxide donor 5-iodo-N- acetylepencilamine (SNAP) for 3 hours, or azidohydidine (AZT) and azidohydidine monophosphate (AZT-MP) from 10 µM to 1 mM for 18 hours. The oxidation of 2',7'-dichlorofluorescein (DCDF, 5 µM) to 2',7'-dichlorofluorescin was assayed by activated cells was measured with a fluorescent plate reader using a 24-well tissue culture plate. Cells were activated by adding 1 or 10 µM of a 10% suspension of polysyllate latex beads per million cells. Determination of hydrogen peroxide produced by non-activated and activated macrophages was based on horseradish peroxidase-mediated oxidation of phenol red.

Results: Latex-activated cells oxidize DCDF due to release of hydrogen peroxide and low-molecular iron complexes, which was verified in experiments using inhibitors catalase (1000 U/ml), desferalex (100 µM) and peroxidase inhibitor sodium azide (2 mM). Preincubation of macrophages with SNAP (18 µM, 3 hours) increased DCDF oxidation by latex-activated cells compared to untreated controls. This effect was absent in macrophages incubated with SNAP depleted of peroxide products for 3 hours. SNAP also increased hydrogen peroxide release by activated cells.

Conclusions: We have observed significant and dose-dependent increases in DCDF oxidation in cells incubated with AZT, or AZT-MP. Catalase (100 U/ml) addition during incubation of cells with latex beads removed the effect of AZT and AZT-MP on DCDF oxidation compared to control cells. Furthermore, AZT and AZT-MP increased hydrogen peroxide release from activated cells.

Authors’ disclosure statement: Supported by NIH R01 HL-65178

The Pharmaceutical Policy In Context Of Health Care System In Albania

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The purpose of this study is to provide an in-depth analysis on the current situation of the Pharmaceutical Sector, to identify the strengths and weaknesses in the sector and also to formulate a proposal document with the main strategic guidelines of Pharmaceutical Policy for present and future reference. My research is focused on some main problems of the pharmaceutical policy in particular it covers pharmaceutical market, general prescribing, public private mix at the pharmaceutical sector, the approved procedures of drugs and medicines, pharmaceutical education and training and drug price and drug distribution.

Background: The environment where the medicines are stored, like drug stores, warehouses and other pharmaceutical distribution centers on the four-membered β-lactam ring, whereas the substituents at the C3 and C4 positions provide certain specificity for recognition by the corresponding enzymes.

Conclusions: A rigid confirmation is a common thread for β-lactamases functioning as enzyme inhibitors. Conformational requirements for recognition by proteases suggest a fundamental platform for the preparation of inhibitors that is dependent on developing conformationally restricted inhibitors which adopt receptor-binding conformation, and are therefore entropically advantaged for binding to a protease. However given this basic requirement for activity, selectivity has been difficult to achieve due to the similarity in the active sites. This double-edged sword will make the future development of inhibitors at once both easier and more difficult because of the potential for inflicting the very damage use of these inhibitors are hoping to block.

Experimental Approach for Growth Inhibition of Human Malignancies by the Highly Efficient Anti-tumor Peptides Delivery System

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Background: Molecular targeting agents have become formidable anticancer weapons, which show much promise against the refractory tumors. Functional peptides are among the more desirable of these nanobio-tools.

Methods: We examined growth suppression efficiencies of aggressive human leukemias/lymphomas, glioblastomas and other human malignancies in vitro and in vivo tumor models by introducing anti-tumor peptides as a complex with the “Wr-T” peptide transporter which serves to augment delivery of a cargo peptide. We did single, or dual peptide introduction of two tumor suppressor peptides (p14[ARF], p21[INK4a], p53, CIP1) or their combinations using Wr-T-mediated intracellular peptide delivery.

Results: Wr-T-mediated transport of p16[ARF] functional peptide dramatically inhibits growth of highly aggressive p16-negative glioblastoma cell lines by up to 80% through restoration of p16 function. Based on this result, we further did Wr-T-mediated simultaneous introduction of two tumor suppressor peptides, p14[ARF] and p16[ARF] functional peptides, into human glioblastoma cell lines, which reversed specific loss of p14 and p16 function, thereby drastically inhibiting tumor growth by >95% within the first 72 h, whereas the growth inhibition was >90% by p14 or p16 single-peptide introduction. Additionally, the combination of p16 and p21[INK4a] peptides dramatically suppressed the growth of glioblastoma line which carries a missense mutation in p53, by 90% after 120 h. Significantly, our murine brain tumor model for dual-peptide delivery showed a substantial average survival enhancement (P < 0.0001) for peptide-treated mice. The similar inhibitory effect by dual peptide introduction was also observed in p16- and p14-double negative cancer cells.

Conclusions: Thus, it was demonstrated that the peptide transporter-mediated delivery of single or dual anti-tumor peptides seems to be highly effective against aggressive human malignancies in non-invasive manner, by singly or jointly restoring multiple tumor suppressor functions.
Energies of Cytochrome P450 Hydroxylations: Making Sense of In Silico:

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Cytochrome P450 catalyzes, among others, the reaction RH + O₂ + H⁺ + NADPH → ROH + H₂O + NADP⁺, in which RH may be a drug. As most drugs interact with cytochrome P450, there are three possibilities: (a) inactivation of cytochrome P450, (b) acceleration of the drug by cytochrome P450, and (c) activation of the drug by cytochrome P450. An ideal drug, or magic bullet, is to be activated, because this is a drug that is inactivated by cytochrome P450 requires an additional drug, namely an inhibitor of cytochrome P450. Use of a pro-drug may indeed be a wise strategy as cytochrome P450 is overexpressed in some cancers. The thermodynamic properties of the intermediates in the cytochrome P450 cycle can be estimated with a few simple assumptions, and the result for the electrode potential of the couple Compound I/Compound II is 1.4 V (W. H. Koppennol, J. Am. Chem. Soc. 129, 9868-9869, 2001). A vast agreement in validation with an estimated ion enthalpy D(Fe-O)=800 cm⁻¹ of ca. 410 kJ/mol (R. T. Green, J. H. Dawson, and H. B. Gray, Science 304 (2004), 1653-1656, 2004). Compound I is almost isosomeric with hammer(III) – hydrogen peroxide complex that precedes it. The electrode potential of 1.4 V is quite different from that implied by the results of ab initio calculations. A higher electrode potential would imply a small association constant between the haem iron(III) and hydrogen peroxide, and a lower value would not allow hydroxylation. Compound I can thus abstract a hydrogen of a primary carbon atom; abstraction from a secondary or tertiary carbon atom is thermodynamically more facile. Thus, hydroxylation to activate a drug is thermodynamically always feasible, and is only limited by the regiospecificity of the hydroxylation. If hydroxylation is undesirable, because it inactivates the drug, then, rather than adding a compound that inactivates cytochrome P450, one might attempt to replace the hydrogen that is abstracted with a fluor atom.

Ab Initio Calculation Of Molecular States Of Compounds Of The Lanthanum And Yttrium Molecules
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The potential energy curves of the grounds and lowest electronic states of the compounds of the Lanthanum and Yttrium Molecules have been investigated via CASSCF method. Multireference CI calculations (single and double excitation with configuration interaction corrections) were performed on the Lanthanum and Yttrium atoms are treated in all electron scheme. In the range of internuclear distance R around the equilibrium distance of their ground states, the molecules are assumed to be mainly ionic. The potential energy curves for the considered states in the representation 1s1 2s0 2p3 have been calculated in the range 2.0 Å < r < 3.5 Å. The spectroscopic constants such as the vibrational harmonic constant uᵢ, the interatomic distance at equilibrium rᵢ, the rotation constant Bᵢ, and the electronic transition energy with respect to the ground state Tₑ have been calculated by fitting the energy values around the equilibrium position to a polynomial in terms of the internuclear distance, the degrees of these polynomials are determined from the evaluation of the statistical error for the coefficients. By using the canonical functions approach and the cubic spline interpolation between each two consecutive points of the potential energy curves obtained from the ab initio calculation, the eigenvalue Eᵢ, the rotational constant Bᵢ, the centrifugal distortion constants Dᵢ, and the abscissas of the turning point (Rₐ, Rₚ) have been calculated for various vibrational levels. The comparison of these values to the theoretical and experimental results available in the literature shows a good agreement. Many electronic states for the considered molecules have been studied theoretically for the first time. All abstracts are listed in alphabetical order of the presenting author.
Electronic Homeopathic Preparations (EHPs) as Potential “Magic Bullet”: Pilot Study on Biologic Model

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Background: Medicinal and diagnostic preparations based on electronic-homoeopathic copying from parent substances have received a certain distribution in complementary medicine in spite of significant deficit of objective scientific papers on their biologic effect. EHP specific action without side effects is declared. The aim is to test the effect of EHP with biological model of tomato seedlings.

Methods: For double blinded randomized trials 4 isotypes of tomato plants re P is to be estimated. It is supposed that electronic-homoeopathic copying phenotype may be related with operator's body being a source of wide-band electromagnetic disturbances associated with the vital activity of cells and organs which are modulated by parent substance during copying.

Conclusions: 1) Revealed in 3 independent double blind randomized trials from 4 isotypes of tomato plants re P it is shown that EHP neutralizes the effect of electronic-homoeopathic copying phenomenon. 2) The multiplicity of effects produced by EHP-re P has been in different trials.

Conclusions: The aim of the present work was to study an antimicrobial resistance of H. influenzae, isolated from throat or nose of 343 healthy preschool children (3-5 years old): 266 children from four day care centers (D group, including subgroups: D1 - 85, D2 - 62, D3 - 44, D4 - 75) and 77 children staying at home (control group, C). For identification of the bacterial isolates routine methods were used. Susceptibility of the tested bacteria to antimicrobial agents was determined by the disc diffusion procedure. Production of beta-lactamases was detected with microcin test.

The prevalence of H. influenzae positive children was 18.18% in C group and 21.05% in D group. 71 isolates of H. influenzae were isolated: 14 from C group and 57 from D group (D1 - 6, D2 - 15, D3 - 7, D4 - 26). 31 (43.66%) of the isolates were resistant to one or more antimicrobials. 7 (50%) from C group and 24 (42.11%) from D group were resistant to ampicillin. The high rate of resistance to trimethoprim/sulfamethoxazole was found - 24% in C group and 29.8% in D group. 8 (11.72%) of the isolates were ampicillin-resistant (C group - 3, D group - 5). 26% of isolates were resistant to amoxicillin/sulbactam. Some of the isolates demonstrated resistance to the other beta-lactams (e.g. II or II’ generation of cephalosporins or monobactams).

The drug resistance pattern of nasopharyngeal H. influenzae isolated from healthy young children may be useful in prediction of drug resistance pattern of H. influenzae of clinical specimens and in consequence, for selection of proper antimicrobial agents used for empiric treatment of respiratory infections caused by haemophilus rods. Besides, these data confirm that drug-resistant strains, being a part of normal microflora, can be considered as a reservoir of resistance genes.

Enveloped Virus Neutralizing Compounds (EVNCs), The Magic Bullets against a Broad Spectrum of Deadly Viruses Causing a Billion Infections Annually Around the globe

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Background: Enveloped viruses like the human Immunodeficiency virus (HIV), influenza viruses including H1N1, hepatitis viruses, HBV and HCV, and herpes viruses 1 and 2 and are currently testing against hepatitis C virus. In order to test the short term toxicity we have used REP and P points to the need for studies on electronic-homoeopathic copying phenomenon. The short term toxicity studies showed that while the unfiltered FA had mutagenic activity, the ultrafiltered FA with 3 kDa balance (Waters Oasis) and have analyzed the fractions for antiviral activity and structural analysis have used the Ames test for mutagenesis. In order to test heat stability we have tested the efficacy and PVT and P points to the need for studies on electronic-homoeopathic copying phenomenon. The short term toxicity studies showed that while the unfiltered FA had mutagenic activity, the ultrafiltered FA with 3 kDa balance (Waters Oasis) and have analyzed the fractions for antiviral activity and structural analysis.

Methods: The aim is to test the effect of EHP with biological model of tomato seedlings.

Results: The prevalence of H. influenzae positive children was 18.18% in C group and 21.05% in D group. 71 isolates of H. influenzae were isolated: 14 from C group and 57 from D group (D1 - 6, D2 - 15, D3 - 7, D4 - 26). 31 (43.66%) of the isolates were resistant to one or more antimicrobials. 7 (50%) from C group and 24 (42.11%) from D group were resistant to ampicillin. The high rate of resistance to trimethoprim/sulfamethoxazole was found - 24% in C group and 29.8% in D group. 8 (11.72%) of the isolates were ampicillin-resistant (C group - 3, D group - 5). 26% of isolates were resistant to amoxicillin/sulbactam. Some of the isolates demonstrated resistance to the other beta-lactams (e.g. II or II’ generation of cephalosporins or monobactams).

The drug resistance pattern of nasopharyngeal H. influenzae isolated from healthy young children may be useful in prediction of drug resistance pattern of H. influenzae of clinical specimens and in consequence, for selection of proper antimicrobial agents used for empiric treatment of respiratory infections caused by haemophilus rods. Besides, these data confirm that drug-resistant strains, being a part of normal microflora, can be considered as a reservoir of resistance genes.

Bacteriologic and therapeutic aspects of paediatrics osteo-articular infections.

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Background: osteo-articular infections included several injuries and interest all the joints bone and the paediatric skeletal. Successful osteo-articular infection treatments need effective antibiotherapy which depends of the causal bacteria and their poor treatment can lead to serious consequences.

Objective: Report the causal bacteria of the osteo-articular infections treated in our department.

Methods: Retrospective study of 1486 osteo-articular infections treated during 18 years has been performed at the general paediatric surgery of Yopougon, Abidjan, Cote d'Ivoire. The age avarage was 7 years a 4, sex ratio was 1.1. The osteo-artitis was observed in 77% and osteomyelitis in 23%. The sickle cell disease was detected in 9% and HIV infection in 0,01%. The first line antibiotic for medical treatment associated coadjuvant (the antibiotic treatment) was observed in 77% of the patients. The second line treatment was indicated when pus collections were documented radiologically. Biologic examinations consist of the blood culture, pus culture, C reactive protein, V and blood count cell. We studied the osteo-articular infection response to the first line antibiotic according bacteriologic characteristic of the causal bacteria (type of bacteria, the bacterial resistance and the antibiogram). Bacteria identification was performed according classic procedure and antibiogram was performed with Oriss Biorad with CASFiM references (committee of antibiogram of French microbiology society).

Results: The blood culture was positive in 31% and the pus culture was positive in 66%. The causal bacteria were, Staphylococcus Aureus in 22%, Streptococcus pneumoniae in 17 %, streptococcus pneumoniae in 13%, Citrobacter freundii in 9%, others bacteria in 20%. The first line antibiotherapy was effective in 25% and antibiotherapy was observed in 75%. The bacteria which produced beta lactase with a large spectrum was observed in 15%, classic quinolone resistance was observed in 15% and the cross resistance to the quinolone in 50%. Staphylococcus aureus was methicillin-resistant in 25%, and negative gram bacteria resistance in 9%. The cross resistance to the quinolone was observed in 50%.

Conclusion: The first antibiotherapy line with oxacillin and thrst generation of cephalosporins indicated osteo-articular infection is less effective because the bacteria resistance.
Anti-microbial, Anti-diarrheal and Toxicity Profile of Cylicodiscus gabunensis Stem Bark (Mimosaceae)

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Background: Diabetes is a major health problem for children worldwide, accounting for 5-8 million deaths each year. Cylicodiscus gabunensis (CG) is a plant of Cameroonian pharmacopoeia. It is reputed for its beneficial effects in the treatment of diabetes related diseases. But their use as medications based simply on a traditional folk use that has been perpetuated among several generations with no scientific data on their efficacy and safety is questionable. Aims: The main objective of this study was to reveal the real efficacy and safety of this drug. Methods: In order to be sure of the therapeutic effects that this plant may have as antidiabetic, the ethanol extract of the stem bark of CG was evaluated in vitro for its anti-microbial

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All abstracts are listed in alphabetical order of the presenting author.

EHRLICH II –2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich
Nürnberg, October 3-5, 2008
Secretin and Autism

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Background: Leading morphological disorders in autism are found in the cerebellum and in the frontal and parietal cortices. In autistic postmortem cerebellum the level of AMPA glutamate receptors, GAD and reelin level decreased compared to control values. Increased homovanillic acid level in the cerebrospinal fluid, hyperserotonemia and endorphinemia, changed serotonin metabolism in the brain are the most consistent disorders in autism. The researchers look for treatments to cure autism. At this moment there are no therapeutic agents which are able to generally improve the autistic phenomena. Insipid of this fact there are many compounds that are used for treating autistic patients and at least in part of the cases they seem to be effective. One of these compounds is secretin. Its significance in autism was first suggested by Horvath and his co-workers (1998, 1999). Their observation facilitated us to explore the occurrence of secretin in the nervous system.

Methods: In our laboratory with the use of immunohistochemistry secretin immunoreactivity was looked for in colchicine treated rats, intact cat and human samples. We have also investigated the role of secretin given intracerebroventricularly (i/cv) on the behaviour of mice with genetic cerebellar atrophy testing open field activity, novel subject approaches and rearing.

Results: Secretin was found in the pyramidal cells of the motor cortex, Purkinje cells, a subpopulation of central cerebellar nuclei, the mesencephalic nucleus of the trigeminal nerve, the superior olivary nucleus, the cells of trapezoid body, a subpopulation of the cells of spinal and trigeminal ganglia. Our data show, that besides a well established synthesis of secretin in the gastrointestinal tract, several nerve structures can produce secretin. icv administration of secretin altered the hypermotility of mice which is characteristic for this genetically modified strain.

Conclusion: Our data and those available in the literature indicate that secretin, besides its gastrointestinal role, is a neuropeptide as well. It was found by Gorszon and D’Antreaux (2003) that the number of secretin producing S cells in autistic patients is half of the normal. Because secretin is present in Purkinje cells, its level has to be dramatically decreased in the case of cerebellar atrophy.

The low level of secretin may be responsible for some autistic symptoms.

Vitamin A and D Derivatives; Potential MAGIC BULLETS with Antithrombotic Applications

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Background: Vitamin (TF) is a member-bound glycoprotein that is essential for activation of the coagulation pathway. Although synthesis of TF is tightly regulated, its expression can be induced by a variety of agonists, such as tumor necrosis factor (TNF) and oxidized low density protein (LDL). Inducing TF expression in endothelial cells, monocytes, or malignant cells is associated with cell motility, migration, angiogenesis, and metastasis. These properties are due to the prothrombotic properties of TF expression. Antithrombotic agents, which are similar in their mechanisms of action, were also investigated. The best MIC and MBC values for the microorganisms sensitive to the extract were 0.00078 and 0.00315 mg/ml respectively. The greater antimicrobial activity of the extract was observed in Streptococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterobacter agglomerans.

Methods: In order to be sure of the therapeutic properties that this plant may have as antidiabetic, the ethanol extract of the stem bark of CG was evaluated in vitro for its anti-microbial activities against 17 pathogenic microbial strains involved in diarrhoea infection isolated from patient: Escherichia coli, Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, Enterobacter freundii, Enterobacter cloacae, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus, Candida albicans and Candida glabrata. Disc diffusion method was employed for the determination of anti-microbial activities and MIC (Minimal Inhibitory Concentration) against the tested strains. The results showed that CG extract exhibited significant anti-microbial activity against various microorganisms sensitive to the extract in vitro. CG extract caused a significant reduction in hepatic malondialdehyde concentrations of aspartate amino-transferase, alanine amino-transferase, total cholesterol and glucose with high-dose of CG treatment tested (3 g/kg). CG also caused a significant reduction in hepatic malondialdehyde concentration. Renal acute and chronic lesions were reduced significantly in test groups. Histological findings revealed a characteristic progression treatment-related effect on liver, kidney and bone. The acute toxicity study was carried out on male Wistar rats weighing 480-520 g. On the day of testing the body weight of the mice was 40-42 g and it was done until 6 weeks and the effects on clinical signs, body weight, food and water consumption, organ weight, haematology, histology as well as serum and renal biochemical parameters were measured.

Results: The best MIC and MBC values for the microorganisms sensitive to the extract were 0.00078 and 0.00315 mg/ml respectively. The greater antimicrobial activity of the extract was observed in Streptococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterobacter agglomerans. Bacillus cereus, Candida albicans and Candida glabrata.

Conclusions: Our data and those available in the literature indicate that secretin, besides its gastrointestinal role, is a neuropeptide as well. It was found by Gorszon and D’Antreaux (2003) that the number of secretin producing S cells in autistic patients is half of the normal. Because secretin is present in Purkinje cells, its level has to be dramatically decreased in the case of cerebellar atrophy.

The low level of secretin may be responsible for some autistc symptoms.

Effects of Intracerebroventricular Injection of Methylprednisolone on Cellulose Oedema after Osmotic Opening of the Blood-Brain Barrier in Rats

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Background: In our work we studied methylprednisolone (MP) for its effects on the permeability of cerebrospinal membranes of neuronal populations in the rat. Methods: We used a standard osmotic cerebrospinal fluid (ICV) model, and administered MP intracerebroventricularly (i.c.v.), as a way of mimicking the effect of TF in leukemic cells or cytokine-stimulated vascular endothelial cells and monocytes. Since RAs and D1 receptors are different in their mechanisms of action we also investigated the anticoagulant effects of D1 and its analogs downregulated TF and upregulated TM expression in monocytic cells, counteracting the effects of TF and oxidized LDL. Purkinje expression of TF mRNA in monocytic cells was markedly downregulated by D1 and its synthetic analogs. We have recently found that D1 suppresses FV expression in monocytic cells by inhibition of AP-1 and NF-kB activation. D1 analogs also effectively downregulated TF in several cancer cells. The more potent D1 analogs, which have far stronger binding affinity to D1 receptor (D1R) have been synthesized.

Conclusions: Several studies report that the D1/D2R system has a physiologcal role in the cell survival of antithrombotic homoeostasis. We propose that the synthetic retinoids and D1 derivatives could be developed as a new type of antithrombotic agent, which will ameliorate the procoagulant character of abnormal cells and act as anticoagulant agents.

Results: In experiment 1 the ID1 values were high (>1), indicating the presence of large quantities of EB in the cells. In experiments 2 and 3 the ID1 values were low (<1), indicating more EB outside than inside cells. ID1 differences between experiments 2 and 1 and experiments 3 and 1 were statistically significant (p<0.05).

Conclusions: In our view, this amounts to morphological evidence of cell membrane integrity restored under the effect of MP.
Fusion Proteins For Flexible Vaccine Antigen Targeting To Cell Surface Receptors

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Background: The initiation of an immune response requires that relevant antigens gain access to the appropriate intracellular compartments to be broken down to peptides, which can then be loaded on major histocompatibility complex (MHC) class I or class II molecules. Peptide presentation by cells providing efficient costimulation is important for priming and boosting T cell responses, a requirement met by dendritic cells (DCs). Targeting of opposing vaccines to antigen-presenting cells is therefore an attractive strategy for eliciting cellular immune responses. However, because of the difficulty of coupling frequently hydrophobic proteins to targeting devices such as antibodies, its potential has been little exploited. This problem could be bypassed by using fusion proteins.

Methods: We have produced several antigens, including hydrophobic viral proteins with an interest as vaccines, as fusion proteins. The soluble proteins also exploited. This problem could be bypassed by using fusion proteins.

Results: Complexes between fusion proteins and suitable antibodies bind specifically to DCs and are internalized into endolysosomal compartments. The fusion proteins are rapidly transported in vivo to CD169+ and later to CD35+ cells in the draining lymph nodes after subcutaneous immunization. Using the model antigen ovalbumin, we show that the fusion proteins, when coupled to various antigens, elicit a humoral immune response and both MHC class I and II restricted T cell responses in vitro and in vivo with an at least 100-fold greater efficiency than antigen alone. Multiple-cytokine-producing CD8+ effector T cells are generated.

Conclusions: 1) The results demonstrate the potential of the strategy for vaccination by initiating a potent immune response. 2) This new tool can be used to stimulate the immune system in a suitable way by targeting the antigen to specific cell surface receptors. 3) A major advantage of this strategy is the possibility to easily compare immune responses by simply exchanging the targeting antibody.

The impact of interferon-β treatment on the blood-brain barrier

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Molecular alterations at the blood-brain barrier (BBB) are essential in the pathogenesis of multiple sclerosis (MS). The implementation of the immunomodulators as the first evidence-based treatment option in the mid 1990s opened a new era in multiple sclerosis therapy. Interferon-β (IFN-β) is commonly suggested to act as an immunomodulator of the cytokine network. However, there is increasing evidence that IFN-β leads to a stearing of BBB in MS patients. This paper will show recent advances in MS with focus on the BBB and will present the author’s contribution on revealing direct and indirect effects of IFN-β on the BBB. It will summarize recent work both with MS patients as well as experimental in vivo and in vitro data. The understanding of IFN-β-derived stabilization of the BBB will not only provide new insights in the pathogenesis of MS but also might be helpful in the development of new, more specifically designed drugs in the treatment of MS.
Effects of Small-volume Resuscitation Using Hypersomolar Saline Colloid Solution on Regional Blood Flow and Ischemic Tissue Injury

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Background: The concept of small-volume resuscitation (SVR) – the rapid infusion of a small dose (4 ml/kg B.W.) of 7.2 – 7.5 NaCl:colloid solution – has been advocated for initial therapy of severe hypovolemia and shock. The solution seems to encompass specific pharmacologic effects, highly relevant for prevention of multiple organ dysfunction syndrome (MODS) induced by trauma and shock.

Methods and Results:

- Hemorrhagic shock

Using the radioactive microspheres method we demonstrated the positive circulatory effect of 7.2% saline given in an amount as small as 1/10 of actual blood loss. In a standardized animal model of severe hemorrhagic hypotension in dogs macrohemodynamic parameters and blood volume were normalized and nutritional blood flow (RBF) recovered within 5 min. RBF was completely restored when combining 7.2% saline with 10% dextran 60 (HHS; hypersomolar-hyporenotropic solution).

- Endotoxin shock

In a pig model of endotoxin shock elicited by continuous i.v. infusion of S. abortus equi leading to a hyperdynamic state with severe circulatory and pulmonary deterioration, 4 ml/kg B.W. of HHS led to significant enhancement of RBF particularly in the small intestine and kidneys.

- Reperfusion injury

Using the hamster dorsal skin-flap for analysis of leukocyte-endothelial interaction HHS proved to ameliorate the activation of polymorphonuclear neutrophils (PMNL). Functional capillary density was augmented and extravasation of macromolecules diminished.

- Lip-regulation of beta2-integrins

In in vitro experiments, hypersomolar saline attenuated N-formyl-methionyl-leucyl-phenylalanine (fMLP) stimulated expression of adhesion molecules on PMNLs. fMLP-stimulated up-regulation of beta2-integrins was diminished.

- Subarachnoid hemorrhage

In a standardized model of subarachnoid hemorrhage (SAH) in rats treatment with 7.5% NaCl plus 6% dextran 70 resulted in lowered intracranial pressure, improved neurological recovery and less morphological damage.

Conclusion: 1) Small-volume hypserosmal colloid resuscitation from severe hemorrhage and shock rapidly mobilizes endogenous water. 2) Nutritional blood flow is enhanced and reperfusion injury diminished. 3) Following subarachnoid hemorrhage hypersomolar saline dextan solution improves neurological outcome.

Molecular Changes Induced by Stress Factors in Cerebral Endothelial Cells

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Being located at the interface of blood and brain, cerebral endothelial cells (CECs) are primary targets of environmental stimuli. The aim of our study was to reveal molecular mechanisms activated by oxidative, hyperosmotic, and calcium depletion induced stress in CECs using an in vitro model of the blood-brain barrier (BBB).

There is increasing evidence that the cerebral endothelium and the BBB play an important role in the hypertension induced brain damage. We have shown that oxidative stress induces a downregulation of the tight junction protein occludin which is more pronounced in the absence of glucose. Furthermore, oxidative stress leads to degradation of the cadherin - catenin complex and an activation of ERK1/2, which is more intense in the absence of glucose. These results indicate that one of the causes of the BBB breakdown is the structural alteration of the junctional complex caused by oxidative stress, a process in which ERK1/2 may play an important role.

Hyperosmotic stress elicited by mannitol has been successfully used to reversibly open the BBB. We have shown that hyperosmotic conditions induce protein phosphorylation on both Ser/Thr and Tyr residues. Among the targets of protein tyrosine phosphorylation is the adherens junction protein beta-catenin. Phosphorylation of beta-catenin on tyrosine residues caused its subcellular redistribution and its dissociation from cadherin and alpha-catenin. All these effects were Src kinase dependent. Osmotic stress is able to induce tyrosine phosphorylation of Axl followed by activation of Akt as well. Moreover, Axl was also cleaved in response to osmotic stress resulting in a 50-55 kDa double degradation product. This process was mediated by a metalloproteinase-dependent cleavage, followed by a proteasomal cleavage. We have shown that besides changes in junctional protein expression and localization calcium removal induces significant changes in the morphological parameters of CECs as well as revealed by atomic force microscopy. These changes could be partially inhibited by the Rho-dependent kinase inhibitor Y27632 suggesting a role for ROCK in mediating the effect of low calcium concentration in CECs.

Our results show that multiple signaling pathways are activated by different stress factors in CECs.

Pharmacokinetic and Pharmacodynamic Modeling of Recombinant Human Erythropoietin

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Erythropoietin is a hematopoietic growth factor stimulating the production of RBC. Recombinant human erythropoietin (rHuEPO) has been indicated for treatment of anemia associated with renal failure, cancer chemotherapy, and HIV antiviral therapy. rHuEPO is an alternative treatment for blood transfusions and its clinical endpoint is to elevate hemoglobin levels. The major clearance mechanism for HuEPO is binding to its receptor (EPOR) followed by internalization and degradation. EPOR are mostly expressed on bone marrow erythroid progenitor cells. Upon binding to its receptor HuEPO initiates several intracellular signaling pathways leading to inhibition of cell death, increase in proliferation, and acceleration of the differentiation processes. All of which causes an increase in number of progenitor cells in bone marrow, and reticulocytes and RBC in blood.

There will be presented several pharmacokinetic (PK) and pharmacodynamic (PD) models that have been developed to describe serum HuEPO concentrations and the reticulocyte, RBC, and hemoglobin responses obtained from clinical trials and studies in animals. The focus will be on applications of concepts target-mediated disposition and lifespan based indirect response in PK/PD modeling of HuEPO. Topics will encompass the nonlinear structure of presented models and stress usefulness of mathematical models in assessment of rHuEPO efficacy and potency. Conclusions will include possible modifications of the PK/PD models to account for neglected so far processes and applications of existing models in development of new protein drugs exhibiting a similar to rHuEPO mechanism of action.
Thiopurine S-methyltransferase and inosine triphosphate pyrophosphohydrolase genes in Japanese patients with inflammatory bowel disease in whom adverse drug reactions were induced by azathioprine/6-mercaptopurine treatment

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Background: The thiopurine S-methyltransferase (TPMT) and/or inosine triphosphate pyrophosphohydrolase (ITPA) gene mutations are suggested to be closely related to adverse reactions induced by azathioprine (AZA)/6-mercaptopurine (6-MP) in Japanese. Screening for mutant alleles may be useful for predicting development of the most serious adverse reactions, agranulocytosis and acute bone marrow suppression.

Methods: Gene mutations for TPMT and ITPA, major AZA/6-MP-metabolizing enzymes, were investigated in 103 healthy Japanese (45 males, 58 females; mean 32.1±13.5 years old) and 16 Japanese patients (9 males, 7 females; 39.1±15.4 years) with inflammatory bowel disease, in whom AZA/6MP treatment induced adverse reactions. TPMT*2 (238G>A) and *3C (719A>G) were analyzed using an allele-specific PCR method, and the TPMT*3A (460G>a), 719A>g, *3B (460G>ta) and ITPA 94a>μ mutations by PCR-RFLP. All experiments were approved by the Ethics Committee of Jikei University School of Medicine.

Results: Analysis of ITPA genes in the 103 healthy subjects showed that 75 (72.8%) were wild-type, while 25 (24.3%) were heterozygous mutations, and 3 (2.9%) homozygous mutations. The frequency of the ITPA mutant allele was 15% (0.150, 95% confidence interval: 0.108-0.206). The TPMT gene was the wild-type in all and the overall incidence of gene mutation detection rate was 50%, with rates of 83.3% in patients with acute bone marrow suppression and 75% in patients with agranulocytosis. The 719A>g allele frequency was 31% (0.313, 95% confidence interval: 0.180-0.486), which was significantly higher than the Japanese standard (chi-square test, p<0.05), while adverse reactions developed earlier in patients with that mutation. In half of the patients, no gene polymorphism was noted, suggesting involvement of drug interactions and reduction of TPMT activity.

Conclusions: In predicting thiopurine adverse reactions in Japanese, it may be favorable to perform screening for the 94a>μ mutation, followed by determination of in vitro TPMT activity when the gene is the wild-type to investigate drug interactions.

Benzimidazoles and surgery in cardiac hydatidosis: efficacy in prevention of disease relapse

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Background: Echinococcosis is a pathology encountered in animal husbandry countries. Cardiac echinococcosis (CE) is a rare entity that might cause serious life-threatening complications. A large number of cases (cystectomy) of hydatid cysts and adjunctive benzimidazole derivatives therapy (BBDT) are still reported. We aimed to analyze case series on surgical correction of CE with adjunctive BBDT therapy as a substudy to our previous study on the recurrence rate of CE in patients treated with BBDT therapy as adjunct to surgery. We aimed to analyze case series on surgical correction of CE with adjunctive BBDT reported in literature and own experience to define the recurrence rate in such combinateive treatment and analyze cases with recurrence of disease.

Methods: We made search in MEDLINE using MESH terms – “cardiac echinococcosis” and “heart diseases” for a period between 1998 and 2008 years. Overall, there were 186 articles retrieved, among them 14 were reporting series (2 to 82) of patients (pts) treated with surgical correction and BDT.

Results: Forty-four studies reported included overall 164 pts with CE (age range 9-75 years, and 88 pts were female). The cyst localization was – left ventricular (LV, including interventricular septum (IVS) aspect) – 37.1% (61 pts, including IVST aspect), right ventricular (RV) – 18.9% (31 pts), IVST – 17.6% (29 pts), right atrial – 9.1% (15 pts), left atrial – 1.8% (3 pts), pericardium – 9.1% (15 pts), sinus of Valsalva – 0.6% (1 pt); 5 pts (3%) had cysts in LV and pericardium, 4 pts (2.4%) – RV and pericardium. Of 164 pts (40.24% pts) had associated lesions in liver (17 pts), lungs (12 pts), spleen (2 pts), and aorta (1 pt). Multiple lesions were found in 8 pts: brain, spine and spleen – 1 pt, brain and kidney – 1 pt, liver and lung - 4 pts, liver and spleen – 1 pt, liver, lung and skin – 1 pt, liver and brain – 1 pt. Surgical removal of hydatid cysts was performed using off-pump cardiac surgery in 47 pts (28.7%) and using cardiopulmonary bypass – 117 pts (71.3%). Therapy with albendazole was started in 81 pts, with mebendazole – in 29 pts. The follow-up period varied between 3 months to 12 years. The CE recurrence rate for albendazole was 3.7% (3 of 81 cases), and 3.4% for mebendazole (1 of 29 pts). The CE relapse occurred in pts taking albendazole with multiorgan, and complicated and complicated lesions (ruptured cysts). Few pts were taking albendazole prior to operation (2 weeks/12 weeks) due to previous lesions in other organs. Cysts characteristics for these pts were distinctive by their solid, univiable and inactive nature, posing less complicated removal during cardiac surgery as compared with cysts at active stage, which might have risk of rupture and dissemination of daughter cysts during surgery.

Conclusion: 1. BDD as adjunct to surgery for cardiac echinococcosis is accompanied by recurrence rate of 3.7%. 2. The CE relapses are related to multiple damage, rupture of cysts and complicated course of disease. 3. Albendazole taken prior to surgery might facilitate the successful surgical removal of cardiac cysts.
Towards a Magic Bullet for the Metabolic Syndrome – Use of the Ginkgo Biloba Extract

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Background: The metabolic syndrome represents a collection of inter-related metabolic defects commonly associated with diabetes (e.g. dyslipidemia, obesity and hypertension) that increase the risk of cardiovascular disease. Ingestion of the dietary supplement Ginkgo biloba Extract might ameliorate the metabolic syndrome by reducing oxidative stress in blood plateletes and the normalizationization of pancreatic beta-cell function in especially T2DM patients with pancreatic exhaustion. Since hyperinsulinaemia is a hallmark of T2DM, it is important to verify that increased insulin production is not due to increased insulin resistance. The primary aim is to measure the effect of the ingestion of Ginkgo biloba extract on whole body insulin sensitivity in subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT, pre-diabetes) and full-blown T2DM.

Methods: Subjects with NGT (n = 10; age, 44.2 ± 13.9 years old), impaired glucose tolerance (IGT) (n = 8; age, 51.3 ± 6.6 years old) and T2DM (n = 8; 51.6 ± 15.2 years old) completed a randomized, double-blind, placebo-controlled crossover study, with each arm lasting 3 months. After ingestion of either Ginkgo biloba extract (120 mg/day as a single dose) or placebo for each arm, a 2-step (each lasting 2 hours) euglycemic insulin clamp was performed, using [2H]glucose intravenous infusion and whole body glucose metabolic rate (M-value) was calculated during the last 30 min of each step.

Results: At the low insulin infusion rate (10 mU/min) the glucose metabolic rates (M-values) were 3.5 ± 1.5 vs. 3.0 ± 0.5 mg/kg (P = 0.16), 3.0 ± 0.4 vs. 2.8 ± 0.8 mg/kg (P = 0.19) and 2.6 ± 0.7 vs. 2.4 ± 0.5 mg/kg (P = 0.09) for the placebo and Ginkgo biloba cycles, in the NGT, IGT and T2DM subjects, respectively. At the high insulin infusion rate (40 mU/min) the M-values were 7.3 ± 2.5 vs. 8.1 ± 2.5 mg/kg (P = 0.07), 6.2 ± 1.6 vs. 6.5 ± 2.1 mg/kg (P = 0.32) and 3.6 ± 1.6 vs. 3.5 ± 1.0 mg/kg (P = 0.34) for placebo vs. Ginkgo biloba cycles, in the NGT, IGT and T2DM subjects, respectively.

Conclusions: The ingestion of 120 mg of Ginkgo biloba extract as a single for 3 months had no significant effect on insulin resistance in non-diabetic subjects or those with pre-diabetes (IGT) nor did it exacerbate the disease in those with full-blown T2DM. Considering the other benefits of ingesting Ginkgo biloba, this is close to a magic bullet as there is fort he metabolic syndrome.

Acknowledgement: This study was supported by NIH grants M01-RR-13.046 and R01-AT-00382 (National Center for Complementary and Alternative Medicine, NCCAM).

New Animal Models for Psychotropic Drug-Drug Interactions

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Background: The involvement of brain neurotransmitters and trophic factors in the mechanisms of psychotropic drug action is commonly accepted. The existing animal models are based on the effects psychotropic drugs on the behavior of animals produced with chronic stress or knock out of the genes involved in the regulation of brain neurotransmission. Here new models for study the role of serotonin and dopamine in psychotropic drug interaction were presented.

Methods: The three new genetic models were created: 1) the ASC/Cba (Antidepressant Sensitive Catalepsy) mouse line selectively bred from a backcross population between CBA/Alt and AKR/J strains for high predisposition to catalepsy. 2) the AKR-CBA-D13Mit76 congenic mouse line with the 61-70 cM brain neurotransmitter (Antidepressant Sensitive Catalepsy) mouse line selectively bred from a backcross population between CBA/Alt and AKR/J strains for high predisposition to catalepsy; 2) the AKR-CBA-D13Mit76 congenic mouse line with the 61-70 cM brain neurotransmitter (Antidepressant Sensitive Catalepsy) mouse line selectively bred from a backcross population between CBA/Alt and AKR/J strains for high predisposition to catalepsy.

Conclusions: The major gene defining predisposition to catalepsy was mapped on the 61-70 fragment of mouse chromosome 13 and linked to the Irmt gene coding the gp130 protein associated with cytokine receptors. The ASC mice showed numerous depressive-like traits and altered serotonin neurotransmission compared with the parental CBA and AKR strains. Chronic antidepressant treatment decreased catalepsy in ASC, but did not alter the trait in CBA mice. The transfer of the 1473G allele of the tph2 gene to the C57BL6J genome significantly affected the interalle gene and depressive-like immobility in the forced swim test.

Conclusion: The ASC mouse line meets face, predictive and construct validity criteria of animal model of depression and antidepressant drugs screening.

Authors’ disclosure statement: The study was supported with Russian Foundation for Basic Research (grant #07-04-00320), Integration Program of the Siberian Division of Russian Academy of Sciences (grant #5) and Russian Program “Molecular and Cell Biology” (grant #10.11).
Anticancer activities of vitamin D analogs

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Background: All-trans-retinoic acid (ATRA) is used for the treatment of acute promyelocytic leukemia as a non-chemotherapeutic drug that induces terminal differentiation of leukemia cells. Another seco-steroid, 1,25(OH)2 vitamin D3 [1,25(OH)2D3] and related compounds also have anti-cancer activities against various types of cancer cells by inhibiting proliferation and inducing differentiation of tumor cells in vitro and vivo. Although orally administered 1,25(OH)2D3 has modest usefulness for patients with myelodysplastic syndrome (MDS) in clinical studies, its use was hampered because of hypercalcemia. 19-nor-1,25(OH)2D3 (Paricalcitol) is approved by the FDA for the clinical treatment of secondary hyperparathyroidism in patients with chronic renal failure. Different from other vitamin D analogues, paricalcitol has very little calcemic potential. This prompted us to investigate its anti-proliferative effect against cancer cells.

Methods: We studied anti-proliferative effect of paricalcitol against cancer cell lines in vitro and in vivo. The combinations of the analog with other clinically useful agents were also tested in vitro.

Results: Paricalcitol has anti-proliferative effects against human cancer cells including prostate and colon cancer cells, as well as leukemia and multiple myeloma cells by inducing differentiation, cell cycle arrest and apoptosis in vitro and in vivo. Among many combinations with other clinically useful agents tested, paricalcitol in combination with arsenic trioxide has markedly enhanced antiproliferative activity against acute myeloid leukemia cells including acute promyelocytic leukemia cells. In further studies, arsenic trioxide acts as an inhibitor of both 24-hydroxylase which is a negative feedback regulator of vitamin D and the PML-RARalpha, the leukemicogenic fusion protein in vivo. This may explain the synergistic effect of the combination against myeloid leukemia cells.

Conclusions: The paricalcitol-calceremic side-effect of 1,25(OH)2D3 has been mitigated by less-calcemic vitamin D analogs such as paricalcitol. The analog and its combination with other clinically useful drugs are being investigated with the hope that they may provide a therapeutic approach to cancers with little toxicity.

Purification and properties of a chemotherapeutic enzyme, L-asparaginase, from Pectobacterium carotovorum MTCC 1428.

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Background: L-asparaginase is widely used in the chemotherapy. However, the success hitherto has been rather limited and most of the treatments have not been so successful due to various allergic reactions. Some of allergic reactions are mainly due to glutaminase contamination of most L-asparaginase. Hence, the discovery of a glutaminase-free L-asparaginase isolated from an organism that is serologically different from the previously reported ones, has similar therapeutic effects will be more advantageous. In this communication we report on the purification and properties of glutaminase-free L-asparaginase extracted from P. carotovorum MTCC 1428.

Methods: The production of L-asparaginase from P. carotovorum was studied in the modified M-9 medium at 30°C and 180 rpm for 12 hrs. The harvested cells were ultracentrifuged and centrifuged at 20000g for 20 minutes at 4°C to obtain crude extract of L-asparaginase. The purification was carried out by ammonium sulfate fractionation (80% saturation), DEAE cellulose ion exchange chromatography and Sephadex G-100 gel chromatography. The various purification steps were examined using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli. A Lineweaver-Burk analysis was used to determine kinetic properties of the purified L-asparaginase.

Results: The L-asparaginase was purified to homogeneity from P. carotovorum cells. Different purification steps (including ammonium sulfate fractionation followed by separation on DEAE cellulose chromatography and Sephadex G-100 gel filtration) were applied to the crude culture filtrate to obtain a pure enzyme preparation. The enzyme was purified 70-fold and showed a final specific activity of 1952 IU/mg with a 39% yield. SDS-PAGE gel showed a single protein band after separation on G-100 gel filtration which revealed the purity of L-asparaginase. Km and Vmax values of purified L-asparaginase was comparable with the existing one which is used as a drug.

Conclusions: This work gives promising results on the possible production of glutaminase-free L-asparaginase which is particularly important for the development of downstream process for efficient production of L-asparaginase from P. carotovorum in a large scale.

Resistance, Including Carbenapenem Resistance, Among Enterobacteriaceae
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Background: Our study was designed to detect enzymes such as ESBLs and AmpC in health-care associated strains of Escherichia coli and Klebsiella pneumoniae, using phenotypic methods and to further characterize carbenapenem resistant isolates. Activity against acute myeloid leukemia cells including acute promyelocytic leukemia cells. In further studies, arsenic trioxide acts as an inhibitor of both 24-hydroxylase which is a negative feedback regulator of vitamin D and the PML-RARalpha, the leukemicogenic fusion protein in vivo.

Methods: To detect enzymes such as ESBLs and AmpC in Escherichia coli and Klebsiella pneumoniae, we used agglutination reaction (ATB) method. The production of L-asparaginase from P. carotovorum was studied in the modified M-9 medium at 30°C and 180 rpm for 12 hrs. The harvested cells were ultracentrifuged and centrifuged at 20000g for 20 minutes at 4°C to obtain crude extract of L-asparaginase. The purification was carried out by ammonium sulfate fractionation (80% saturation), DEAE cellulose ion exchange chromatography and Sephadex G-100 gel chromatography. The various purification steps were examined using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli. A Lineweaver-Burk analysis was used to determine kinetic properties of the purified L-asparaginase.

Results: Of 126 isolates that were resistant to third generation cephalosporins (39 E. coli and 87 K. pneumoniae) in 2005, we found AmpC in 28.2% (11 isolates) of E. coli and in 17.2% (15 isolates) of K. pneumoniae. In addition 8 isolates were resistant to carbenapenems (5 isolates of K. pneumoniae and 1 isolate of E. coli). A combination of phenotypic tests revealed a likely co-existence of AmpC and ESBL in both E. coli and K. pneumoniae. Resistance to cefepime was observed in 83% of phenotypic AmpC positive isolates (10 out of 12 tested isolates). Multiple ß-lactamases were detected in K. pneumoniae and E. coli with reduced susceptibility to carbenapenems. The presence of carbenapenemases was suspected in isolates with reduced susceptibility to carbenapenems, using phenotypical methods. A single isolate of K. pneumoniae was suspected to harbour a carbenapenemase and a MBL, using phenotypical methods. K. pneumoniae with reduced ß-lactamase activity harboured carbenapenemases were susceptible to aminoglycosides and trimethoprim sulfamethoxazole.

An increase in resistance to carbenapenems in E. coli isolates was observed in the hospital between 2005 and 2007 while a decrease was observed in K. pneumoniae.

Over all resistance against cefepime was 24.6% in 2007 for E. coli and 39.3% for K. pneumoniae. There were no differences in resistance to ceftriaxone and cefotaxime indicating that AmpC carrying isolates are still rare in the hospital population. However, among isolates from both species there was reduced sensitivity to imipenem.

Conclusions: Results of phenotypic tests for resistance properties may be helpful. Further work is being done to compare the results of phenotypic tests with the outcome of genotypic investigations.

Missile Injuries of Orofacial Region, Primary and Secondary Phase Management

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Background: In a society struggling to rebuild the country after three decades of years of dictatorship and wars, Iraqi craniofacial and maxillofacial surgeons play a critical role in treating many of most serious facial injuries. Of orofacial region by on going conflict in Iraq. This study reflects the modern and advanced surgical techniques of treating explosive injuries and other combat and terrorism related injuries and also evaluate the immediate phase and secondary phase management of 167 patients suffering from missile injuries.

Methods: This study includes one hundred sixty seven patients with missile injuries of orofacial region in a period of 4 years, all injured patients were treated at maxillofacial units.10th floor, surgical specialties hospital, medical city Baghdad. There were 134 men and 33 women, the age ranged from 9 to 70 years (mean 39.5 years) of age.

Result: In addition to 27 patients with orbital injuries, they were 134 patients men and 33 women; their age ranged from 9 to 70 years (mean 39.5 years).

Orofacial deformities classified into following: 1-soft tissue; 2- bone loss; 3- bone loss 2-soft tissue loss; 4- twenty seven patients (16.16%) with orbital injuries 4- thirty patients(17.96%) had other deformities' of scar contraction, fistula and sinus formation.

The bony defect was reconstructed by both bone chips carried by osteosynthetic tray harvested from the iliac crest and by block of cortico-cancellous bone graft from the iliac crest. Soft tissue reconstruction done by local flaps and regional flaps such as lateral cervical and cervico-facial flaps and orbit reconstructed by bone graft, lyophilised Dura and silicon template. Scar contracture treated by scar revision and sinus tract excised at the same time of scar revision.

Conclusions: Primary phase required an urgent airway management, controlling active bleeding by surgical intervention; most entrance and exit wounds as well as retained missile were located in the cheek, chin and mandibular body with few cases of mortality due to complication related to head injuries. Secondary phase management of deformities of the face as a complication of missile injuries were classified as bone loss, soft tissue loss, combined bone and soft tissue loss and others(sinus tracts and poor scars).
Serum hepcidin level (HEPC) is a significant predictor of arterial stiffness in maintenance hemodialysis patients (mHD)

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Background: We have already demonstrated that HEPC were significantly higher in mHD than control. Furthermore, in multiple regression analysis, only ferritin (β=0.514, P=15.78, p<0.0001) was selected as a significant predictor of HEPC. Recently, HEPC has been suspected to be linked to the cause of anemia of inflammation and cardiovascular disease (CVD). It has been well known that pulse wave velocity (PWV) is a predictor of CVD. For the purpose of clarifying the relationships among HEPC, iron metabolism and CVD, we evaluated HEPC, indexes of iron metabolism, risk factors of CVD, and brachial-ankle (ba-PWV) in mHD.

Methods: 198 mHD, who were treated with erythropoietin and 33 healthy controls were recruited in this study. Hemoglobin (Hb), [2-microglobulin (MG), calcium (Ca), phosphorus (P), intact-parathyroid hormone (int-PTH), total cholesterol (T-CHO), triglyceride (TG), iron, HEPC, ferritin, total iron binding capacity (TIBC), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and ba-PWV were also measured. HEPC was measured by liquid chromatography tandem mass spectrometry.

Results: Serum levels of TNF-α(27.1±0.7 vs. 5.3±0.7 pg/mL; p<0.001), IL-6 (12.4±1.2 vs. 3.6±1.1 pg/mL; p<0.001) and HEPC (44.8±2.9 vs. 4.4±3.7 ng/mL; p<0.001) were significantly higher in mHD than control. ba-PWV was significantly correlated with TNF-α(0.27, r=0.072, p<0.001). Likewise, control (0.28, r=0.008), but not with IL-6, duration of HD, KSV, Ca, P, int-PTH, (2-MG), T-CHO, or TG. In simple regression analysis, TNF-α was identified as a significant determinant of ba-PWV (β=0.26, t=1.58, p<0.001) while other variables were not selected as significant predictors of ba-PWV in mHD. These finding show that iron metabolism and inflammation might affect arterial stiffness, which could link to CVD in mHD.

Conclusions: We believe that HEPC could be a useful biomarker for cardiovascular disease in maintenance hemodialysis patients.
For Prion's Pathogenic Conversion Reaction

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Background: Prion proteins are key molecules in transmissible spongiform encephalopathies (TSEs). Although the precise mechanism of the conformational conversion process from the cellular form (PrP\textsubscript{C}) to the scrapie form (PrP\textsubscript{Sc}) is still unknown, we demonstrated that it is totally feasible to design a chemical chaperon which can stabilize the PrP\textsubscript{C} conformation, and regulate the conversion reaction. To design the chaperon, we utilized the slow dynamical information of a prion protein, and concurrently identified the hot spots for pathogenic conversion reaction.

Methods: We conducted in silico screening to find compounds that fitted into a “pocket” created by residues undergoing the conformational rearrangements between the native- and the sparsely populated high energy states (PrP\textsubscript{C}*) directly bind to those residues. Hit compounds were tested by ex vivo and in vivo screening, and if effective, they were subjected to determination of the complex structure and further lead optimization processes. The cyclic process between the native- and the sparsely populated high energy states (PrP\textsubscript{C}*) was repeated recursively.

Results: More than hundred compounds were tested in a TSE-infected cell culture model, and more than twenty compounds including, 2-pyromellitil-1-yl-N(4-fluor-2-pyromellitin-1-yl-acetylamino)-benzyl(phenyl)acetamide, termed GNI8, efficiently reduced PrP\textsubscript{Sc}. Subsequently, administration of GNI8 was found to prolong the survival of TSE-infected mice. Heteronuclear NMR and computer simulation showed that the specific binding sites are the A-S2 loop (N159) and the region from helix B (V189, T192 and K194) to B-C loop (E196), indicating that the intercalation of these distant regions termed “Hot Spots” hampers the pathogenic conversion process.

Conclusions: Dynamics Based Drug Discovery (DBDD) strategy demonstrated here focusing on the hot spot of PrP will open the way to the development of novel anti-prion drugs.

Oral Tolerance as a Method of Suppression of Immunological Response in Experimental Autoimmune Encephalomyelitis

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Background: Recently has been proposed to apply a method of oral tolerance to ameliorate auto-immune reactions. The aim of this study was to use the hydrolysate of pig spinal cord proteins (mixture of neuroantigens) to induce oral tolerance in the animal model of sclerosis multiplex (SM) - experimental allergic encephalomyelitis (EAE).

Methods: The female Lewis rats were fed with pig spinal cord hydrolysate in two doses for one week before immunization, which was induced by injection of guinea pig spinal cord homogenate. The clinical course was observed and evaluated in a five grade scale. At the peak of clinical symptoms (the 13th day post immunization) the rats were sacrificed and the spleen removed. Splenocytes were suspended in a culture medium and placed in microculture plates. The cells were stimulated with homogenate alone, hydrolysate alone, mixture of homogenate + hydrolysate, and medium alone. The cells were cultured for seven days. Subsequently, proliferation of splenocytes was estimated by means of [\(^3\)H]thymidine incorporation and expressed in cpm (average of triplicate samples).

Results: It was demonstrated that homogenate-induced splenocytes of hydrolysate-fed rats gave rise to low proliferation as compared to the controls used. The IFN-\gamma was inhibited in hydrolysate-fed animals as well as in hydrolysate-stimulated samples.

Conclusion: The results show that the hydrolysate of pig spinal cord proteins has a modulatory effect on the immune reaction, particularly on the orally-induced antigen-specific modulation of autoimmune response. It might have a clinical implication in SM treatment.
Interactions Between Drug Target Binding Sites and the Remarkable Story of Dopamine D1/D2 Synergism

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Background: Biological effects elicited by concomitant binding of two or more drugs to distinct but interacting proteins identifies these binding networks as potential drug targets. For example, recent findings on ligand-activated receptor dimerization and receptor-G protein-accessory protein coupling suggest a potentially useful approach based on Boolean logic: "AND" operations lead to fewer possible outcomes than "OR" operations. Translation of these concepts to biological processes gives rise to the concept of "Magic Bullet Cocktails." Twenty years ago, a remarkable discovery about signal transduction pathways in the brain was made, namely that the widespread effects of the neurotransmitter dopamine, with few exceptions, require concomitant agonist stimulation of both D1 and D2 receptor subtypes, a phenomenon referred to as "D1/D2 Synergism." Even more remarkable is the fact that shortly after depletion of synaptic dopamine, there is a breakdown in synergism: all of the effects of dopamine can be elicited by drug stimulation of either D1 or D2 receptors which are now profoundly supersensitively to stimulation.

Methods: We have used receptor autoradiography, behavioral analysis, differential display, and gene knockout techniques to identify the mechanism(s) of D1/D2 synergism and its breakdown.

Results: First, we showed that increases in dopamine receptor number cannot account for these changes. In order to identify unknown candidate genes whose expression may contribute to these phenomena, we used Differential Display of mRNA, which led to the discovery of a novel transcript later identified as rhps, a gene encoding a Ras homolog that functions as a G protein accessory protein. We then showed a breakdown in synergism and produced supersensitively consistently result in decreased expression of rhps mRNA and Rhps protein. Furthermore, Rhps knockout mice are supersensitive to D2 but not D1 receptor agonists. Thus Rhps may normally serve to inhibit D2-mediated signaling.

Conclusions: A Magic Bullet Cocktail consisting of a drug that facilitates the action of Rhps in combination with a D1 and a D2 antagonist should provide a novel treatment for schizophrenia that is superior to existing therapies.


La Rocca G1, Anzalone R1, Capeello F2, Corrao S3, Timperio AM4, Zolla L, Conny De Macario E1, Macario A5, Farina F6, Zummo G7

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Background: Hsp10 expression has been investigated in several cancer models, with contrasting results. It is homologue to early pregnancy factor (EPF), a secreted protein which modulates the immune response of the mother versus the fetus. The impact of cigarette smoke (a major risk factor for lung diseases) on Hsp10 expression by airway cells has not been characterized yet.

Methods: We studied the effects of non-lethal doses of cigarette smoke extract (CSE) on the expression of Hsp10 and Hsp27 in human lung cells. Proteomics was performed in both untreated and exposed cell lines, western blotting, and mass-spectrometry (MS). Database searches and chaperonomics were used to identify the proteins and genes of interest.

Results: Following CSE cell exposure as compared with unexposed cells, significant variations in Hsp10 did occur. In both lung fibroblasts and epithelial cells. In unexposed cells, three isolectric variants of Hsp10 were found, which have not been reported for any other system, yet. After CSE exposure, only the most basic isofrom was still expressed. To characterize the three variants found in unexposed cells, we performed MS analyses. Digested spots were analysed by nano-RP-HPLC-ESI-MS/MS to determine the fragments’ amino acid sequences. Database searches showed that the most basic variant was human Hsp10 with 56% sequence coverage, and the other two isoforms had the same amino acid sequence, even if with a lower sequence coverage.

Conclusions: This data thus far indicate probably that Hsp10 protein variants are due to post-translational modifications. We recently showed the in vivo correlation between lung cancer development and downregulation of Hsp10 expression, and proposed a model for the antitumoral role for Hsp10, together with Hsp27. The precise role of Hsp10 in carcinogenesis is still unclear. The immunosuppressive activity of EPF/Hsp10 points toward a possible role, mediating immune evasion and apoptosis resistance. On the other hand, the in vivo and in vitro evidences obtained in human lung models suggest that different Hsp10 isoforms may mediate diverse processes and should be differentially regulated.

Polypharmacy: A Major Risk In The Life Of The Elderly

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Addressing the high utilization of medications among the elderly, this study explored the degree of knowledge of polypharmacy and its consequences, as well as the pattern of utilization by a group of elders 65 years of age and over in the San Juan metropolitan area of Puerto Rico. A dual technique was utilized: focus groups and a survey. Two focus groups were performed in order to explore issues pertaining the following domains: quality of life, doctor-patient and pharmacist-patient relationships, degree of knowledge of polypharmacy, and need of information. A questionnaire was administered to obtain information regarding their pattern of drug utilization. Results: Focus groups indicated that the elderly have learned how to cope with their chronic conditions and are able to continue with quality of life. Additional results suggest a need form the elderly to develop more assertiveness and closeness with their health care providers, both doctor and pharmacist, as well as a lack of sufficient knowledge of the serious implications that polypharmacy brings. Results from the survey revealed and confirmed their inappropriate utilization of medications, and suggest that this segment consults multiple physicians, all of which may bring potential problems of adverse drug interactions. Further investigation is needed to examine fully the issue of polypharmacy, as well as the need of education that results in empowerment of patients, families and communities.
Bioresorbable Nanoparticles, Carriers Of The Magic Bullet

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Background: Current barriers to cancer chemotherapy include i) toxic side effects, ii) the limited accessibility of the drugs to tumor tissue, and iii) drug-resistance development by malignant tumors during treatment. We developed a novel drug delivery system utilizing reconstituted high density lipoprotein (rHDL) nanoparticles with reduced toxicity to normal tissues and selective receptor-mediated uptake of anti-cancer drugs. The HDL formulation is preferred over conventional drug delivery strategies because its small size and biocompatible components. A further advantage of the rHDL drug delivery model is the selective lipoprotein uptake mechanism by which drugs are delivered to target cells via the scavenger receptor, class B, Type I (SR-BI).

Methods: The rHDL/paclitaxel (PTX) nanoparticles were characterized regarding to size, shape, stability and cytotoxicity against cancer cells when compared to free PTX. The majority (82%) of the paclitaxel was taken up by cancer cells via a selective uptake mechanism, apparently via the SR-BI receptor. Incubation of the cells with HDLs, the natural ligand of SR-BI, suppressed paclitaxel uptake to 30.6% as compared to HDL/PTX alone (p<0.001) supporting the specificity of the receptor uptake mechanism. During studies with mice a 2.3-fold and 1.4-fold higher dose of HDL/PTX could be tolerated by mice, compared to Taxol® and Abresol®, respectively. Recent tumor suppression studies with mice show that the HDL delivery system is highly effective in reducing the tumor burden in mice carrying xenografts of human tumors.

Results: The rHDL/PTX nanoparticles were found to have a diameter of 11.4 +/- 3.1 nm and 5-20 fold enhanced toxicity against cancer cells when compared to free PTX. The majority (82%) of the paclitaxel was taken up by cancer cells via a selective uptake mechanism, apparently via the SR-BI receptor. Incubation of the cells with HDLs, the natural ligand of SR-BI, suppressed paclitaxel uptake to 30.6% as compared to HDL/PTX alone (p<0.001) supporting the specificity of the receptor uptake mechanism. During studies with mice a 2.3-fold and 1.4-fold higher dose of HDL/PTX could be tolerated by mice, compared to Taxol® and Abresol®, respectively. Recent tumor suppression studies with mice show that the HDL delivery system is highly effective in reducing the tumor burden in mice carrying xenografts of human tumors.

Conclusions: Reconstituted high density lipoprotein (rHDL) provides a targeted delivery vehicle for the encapsulated paclitaxel via receptor mediated uptake of the drug by cancer cells and tumors. Encapsulation of chemotherapy drugs in HDLs enhances the systemic clearance of the drug while increases its toxicity against cancer cells and tumors. The rHDL nanoparticles should thus reduce the toxic side effects seen with other formulations while enhancing the anti-tumor effectiveness of the encapsulated drug.

Desiring Novel Antiinfective Concepts Combining Nanotechnology, Bioplastics and Natural Products

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Abstract: This presentation reviews a number of research efforts carried out within our group where combining natural products, nanotechnology and bioplastics respectively. Recent advances in both the production of novel efficient antiinfective systems. The talk presents first our recent efforts to understand and optimize the antimicrobial properties of chitosan and electrospun nanofibrous chitosan and of other antimicrobial biomass derived biopolymers and blends (see Figure 1). It does describe our most recent efforts to design antiinfective and bioactive bone replacement interphases and wound dressing systems based on nanostructured fiber mats of biopolymers carrying biocides and carried out within the EU FP6 project NEWBONE. Finally, the talk presents describes the capacity of certain nanomaterials to deliver bioactive and control release biocide and bioactive plant extracts (see Figure 2) and, within a very recent collaboration with the University of British Columbia and Riose DTU, of pharmaceutical antibiotics such as tetracycline.

Figure 1. Antiinfective nanostreamed blend of electrospun PLA-chitosan

Selection Of Cell Culture Substrate For Human Viral Vaccines

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Background: Many cell cultures for human viral vaccines used up today, animal origin or human diploid cells (Wi-38), primary culture or continued cell lines like VERO. The rabies vaccine produced on BHK-21/C-13 (Baby Hamster Kidney) cell culture has been used for a long time in animals. Since the safety of the BHK cells as an animal origin is not questionable area to develop novel efficient antiinfective systems. The talk presents first our recent efforts to understand and optimize the antimicrobial properties of chitosan and electrospun nanofibrous chitosan and of other antimicrobial biomass derived biopolymers and blends

Methods: We multiplied L. Pasteur strain of rabies virus on the BHK-21 (C-13) cells and produced beta-propiolactone inactivated and aluminium-phosphate adsorbed rabies vaccine. A total of 300 adult subjects were vaccinated. A clinical testing was conducted with three doses of vaccine, intramuscularly in the deltoid region by the pre-exposure scheme 0-7-21 days. At 30th day post vaccination serum antibodies were measured by the RFFIT (Rapid Rabies Focus Fluorescence Inhibition Test).

Results: In comparison with VERO cell line of monkey origin, we harvested one log more rabies virus from BHK cells and vaccine production is possible without virus concentration. Local reactions in few percents and no sistemic adverse reactions were registered. All vaccinees had antibody titer over acceptable minimal (0.5 UI). We also adapted polio and measles viruses on BHK cells for possible vaccine production.

Conclusions: We conclude that this rabies vaccine is low cost, safe and effective for humans. The preliminary results with BHK-21 vaccine in volunteers confirmed its good tolerability and immunogenicity. This product described is safe on the basis of experimental results that virus inactivation by beta-propiolactone destroys contaminant DNA from cell culture.

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Abstracts

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target cancer cell is lysosomally processed to release the thioether-linked stability of the conjugate and to facilitate the intracellular release of the hindrance at carbon atoms adjacent to the disulfide bond to maximize plasma non-reducible thioether link. The disulfide linkers are designed with steric molecules are linked to the antibody molecule at lysine residues via a disulfide or a press.

Clinical Validation of Maytansinoid Technology: Currently eight AMC compounds are in clinical testing using ImmunoGen’s maytansinoid technology, with one more expected to enter the clinic in 2008. One of the compounds in the clinic, T-DM1, is a conjugate of the maytansinoid DM1 with the antibody trastuzumab. It is being evaluated in patients with HER2-expressing metastatic breast cancer that have progressed on treatment with a chemotherapy regimen that includes trastuzumab. The initial clinical data reported is encouraging, and includes a confirmed objective response rate of 44% (4/9) at a dose of 3.6 mg/kg q3weeks in a Phase I trial in such trastuzumab-nonresponsive patients.

Understanding the Mechanism of Cell Killing by AMCs: The maytansinoid molecules are linked to the antibody molecule at lysine residues via a disulfide or a non-reducible thioether link. The disulfide linkers are designed with steric hindrance at carbon atoms adjacent to the disulfide bond to maximize plasma stability of the conjugate and to facilitate the intracellular release of the maytansinoid by disulfide-reduction. The non-cleavable thioether link is designed to be stable in plasma, and the conjugate upon binding and internalization in the target cancer cell is lysosomally processed to release the thioether-linked maytansinoid attached to the lysine residue.

Broadening the Technology: Evaluation of the role of linkers in the effective intracellular cytotoxic maytansinoid metabolites has led to the creation of novel hydrophilic linkers that are stable in plasma and yield even greater efficacy to the conjugates based on in vitro and in vivo pre-clinical studies. The hydrophilic linkers confer improved activity against multi-drug-resistant (mdr) cancer cells, and also offer potential for agents against tumors that express the target antigen at low density.

Clinical Validation of Maytansinoid Technology:

Understanding the Mechanism of Cell Killing by AMCs:

Broadening the Technology:

Magic Bullets And Vaccines: Learning From The Brain

LAMBERT JM ImmunoGen, Inc., Waltham, MA, USA

Background: In recent years, there has been increasing interest in the use of highly toxic small molecules as the attached cell-killing agent. One reason for the heightened interest in the field is the increase in the number of companies pursuing antibody-based anticancer agents, since many tumor-targeting antibodies identified as a result of this effort lack meaningful anticancer activity of their own. Creating antibody-maytansinoid conjugate (AMC) compounds provides a means of achieving effective products from such antibodies.

Clinical Validation of Maytansinoid Technology: Currently eight AMC compounds are in clinical testing using ImmunoGen’s maytansinoid technology, with one more expected to enter the clinic in 2008. One of the compounds in the clinic, T-DM1, is a conjugate of the maytansinoid DM1 with the antibody trastuzumab. It is being evaluated in patients with HER2-expressing metastatic breast cancer that have progressed on treatment with a chemotherapy regimen that includes trastuzumab. The initial clinical data reported is encouraging, and includes a confirmed objective response rate of 44% (4/9) at a dose of 3.6 mg/kg q3weeks in a Phase I trial in such trastuzumab-nonresponsive patients.

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Conclusion: The pharmacokinetic and clinical validation of the guidelines developed suggests that they are efficient and safe for the initial dosing of gentamicin in term and premature babies.

Clinical Guidelines For The Use Of Extended Interval Dosage Regimens Of Gentamicin In Neonates

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Background: Development and validation of guidelines for gentamicin dosing in neonates using extended-interval dosage regimens.

Methods: With the base of previously obtained pharmacokinetic parameters (JM Lanao, JAC 48:1038-48, 2004), dosing guidelines were designed to achieve serum gentamicin concentrations (SGCs) within the ranges considered therapeutic in adults for extended-interval dosing (peak 15-20 mgL and trough <0.5 mgL). These guidelines were adopted as the dosing practice at our institution.

Results: gentamicin population comprised 81 neonates dosed according to the proposed guidelines, routinely monitored, with the following clinical characteristics: gestational age (GA) 24-40 weeks (mean SD): 30.48 (4.42), and postnatal ages of 1-11 days (2.80 (1.52)). C-reactive protein (CRP) and serum creatinine were measured at the start and end of treatment for the evaluation of efficacy and toxicity of the treatment.

Results: In term newborns and premature babies with GA between 31-38 weeks, extended-interval dosage regimens with initial gentamicin dosages of 10-12 mg/kg and dosage intervals of 36-48 h are recommended. Owing to their high distribution volumes and prolonged half-lives, for premature babies of GA <31 weeks we recommend initial doses of 5 mg/kg and dosage intervals of 36-48 h to reach SGCs between 0.5-10 mg/L.

A linear relationship between the individualized dose after SGCs monitoring (ID) and guideline dose (GD) was obtained: ID = 0.9469 GD - 0.909; r = 0.8991. A statistically significant difference (p=0.00) was found between initial and final CRP levels in patients with sepsis (2.45 (1.38) vs 1.29 (1.56)mg/dl) or suspected infections (2.041.85) vs 0.87 (0.26) mg/dl). A statistically significant decrease in the serum creatinine concentration was also observed (p<0.01).

Conclusion: The pharmacokinetic and clinical validation of the guidelines developed suggests that they are efficient and safe for the initial dosing of gentamicin in term and premature babies.
Erythropoietin in Cancer Anaemia: Friend or FoE?

LAPPIN TRJ

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Erythropoietin (EPO), a glycoprotein hormone produced mainly in the kidney and the liver, binds to the EPO receptor (EPO-R) on erythroid precursor cells in the bone marrow, thereby promoting their survival, proliferation, and differentiation. In adults normal erythropoiesis produces about 2.3 million red blood cells per second, regulated by basal levels of 0.8–4.0 picomoles/L of EPO (5–25 U/L) in plasma.

Many cancer patients suffer from anaemia, and recombinant human EPO and other erythropoiesis stimulating agents (ESAs) are widely used therapeutically, to increase haematocrit, lower blood transfusion requirements, and improve quality of life. However in recent years, several investigators have identified EPO-R expression in numerous cancers and tumour cell lines, raising concerns about the safety of ESA therapy for cancer-associated anaemia. Pharmacological doses of EPO elevate plasma concentrations several fold and potentially could modulate tumour growth.

In 2006, a Cochrane Review collated data on over 9000 cancer patients from 57 trials in which recombinant EPO or darbepoetin alfa was given to prevent or treat anaemia. ESA-treated patients had significantly lower blood transfusion requirements. Although there was no significant difference in survival between ESA- and placebo-treated patients, none of the trials included in the meta-analysis satisfied the eligibility criteria. Despite this, no significant differences were found between ESA-treated patients compared with controls overall survival. The relative risk for thromboembolic events was much higher in ESA-treated patients compared with controls. EPO elicits plasma concentrations several fold and potentially could modulate tumour growth.

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Vitamin C In Intravenous Nutritive Solution: Double-Edge Effect For Premature Newborn Infants

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Background: Premature infants are at risk of pathological complications related to oxidative phenomenon. Among them, the incidence of broncho-pulmonary dysplasia (BPD) is near of 40% in newborns of < 28 weeks of gestation. Because of the immaturity of their gastrointestinal system, these children are frequently nourished by intravenous way. Although this parenteral nutrition (PN) contains antioxidant vitamins such as vitamin C, this solution is contaminated by peroxides. This contamination is associated with inadequate proto-protection of nutritive solution. The interaction, catalyzed by photo-excited riboflavin, between vitamin C and oxygen dissolved in solution generates hydrogen peroxide (H₂O₂). The infusion of PN without photo-protection to newborn guinea pigs induces a lower amount of alveol in lungs, a characteristic feature of BPD. This observation is not induced by an infusion of H₂O₂, vitamin C or riboflavin alone, but with the combination of vitamin C + riboflavin. The interaction between dehydroascorbate and H₂O₂ generated in PN leads to the formation of a new compound, named ascorbylperoxide (2,3-diketo-4-hydroxyperoxyl-5,6-dihydroxyhexanoic acid). We hypothesize that ascorbylperoxide is the active agent leading to a low alveoli development in newborns.

Methods: Three days old guinea pig pups received intravenous solution containing increasing concentrations of ascorbylperoxide. After 4 days, lungs were sampled for histological determination of alveoli. Ascorbylperoxide concentrations in PN as well as in urine samples were determined by mass spectrometry.

Results: The alveoli count was negatively correlated (r² = 0.96, p<0.01) with urinary logarithmic concentration of ascorbylperoxide. The addition of glutathione into PN solution allows the recycling of DHA in ascorbate, thus preventing the generation of ascorbylperoxide and degradation of vitamin C.

Conclusion: Results suggest that the interaction of ascorbate with other components present into PN such as riboflavin and oxygen contributes greatly to development of BPD in premature infants. The addition of glutathione to PN could prevent the loss of alveoli and improve the availability of vitamin C.

Supported by Canadian Institutes of Health Research (MOP 79403)

Folate-Targeted Chemotherapy


Endocyte Inc., West Lafayette, USA

Background: The small molecular weight ligand, folic acid, is capable of targeting covalently attached bioactive agents quite specifically to folate receptor (FR)-positive cancers. To date, impressive anti-tumor activity has been observed preclinically with folate conjugates of potent chemotherapeutic molecules, and a few of these agents have recently entered the clinic with more to soon follow. In this preclinical investigation, we explored the possibility of using the folate ligand to target a potent, semi-synthetic analog of the microtubule inhibitor, tubulysin B, to FR-enriched tumors.

Methods: EC0305, a folate-tubulysin conjugate, was evaluated in vitro for dose-dependent cytotoxic activity against a panel of FR-positive and negative cells. Cell lines selected for EC0305 were cultured for 2 h in the presence and absence of excess folate, and then chased in fresh medium up to 72 h. N/n/mice (Baltic background) were inoculated with FR-positive KB cells, and 74 ± 14 mm tumors were established 11 days later. EC0305 was then administered through the lateral tail vein, and tumors were measured every 2-3 days using a caliper. Tumor volumes were calculated and then compared to untreated controls.

Results: EC0305 was found to specifically inhibit the growth of a panel of FR-positive cell lines (IC₅₀ range 1 to 10 nM) in a dose-dependent manner, whereas cells lacking FR expression were unaffected. EC0305's potency was also confirmed against a human KB xenograft-nu/mouse cancer model. Here, a brief three times per week, 2 week regimen yielded remarkable anti-tumor activity (100% tumor-free animals) without causing significant weight loss or major organ tissue degeneration. In contrast, anti-tumor activity was completely abolished in EC0305 mice that were co-dosed with an excess of a non-toxic folate-containing analog, thereby confirming that this agent's anti-tumor effect was mediated by FRs. The advantage provided by folate conjugation was further proven by the un-targeted free drug, which was found to be completely inactive at both tolerable and highly toxic dose levels.

Conclusions: These results collectively show that a potent anti-proliferative tubulysin compound can be specifically delivered to FR-positive tumors to provide substantial therapeutic benefit using well-tolerable dosing regimens.

Hydrophilic hexapeptides – a new class of ATF – dependent transport proteins of multiple drug resistance

LEBEDEV VV

Central Research Institute of Epidemiology, Russia, Moscow.

Multiple drug resistance (MDR) appears as a result of short rise in pumping out medical products from a cell into extracellular space by ATP – dependent transport proteins. At present no transport protein effective inhibitor is available that could be used for the MDR overcoming. The effect of three hydrophilic hexapeptides, including cyclic hexapeptide on activity and amount of transport proteins responsible for MDR formation has been investigated. Cell lines of human throat cancer, Hep2, human oral cavity carcinoma, KB 8-5, and human prostate cancer, LNCaP were established 11 days later. EC0305 was then administered through the lateral tail vein, and tumors were measured every 2-3 days using a caliper. Tumor volumes were calculated and then compared to untreated controls.

Results: EC0305 was found to specifically inhibit the growth of a panel of FR-positive cell lines (IC₅₀ range 1 to 10 nM) in a dose-dependent manner, whereas cells lacking FR expression were unaffected. EC0305's potency was also confirmed against a human KB xenograft-nu/mouse cancer model. Here, a brief three times per week, 2 week regimen yielded remarkable anti-tumor activity (100% tumor-free animals) without causing significant weight loss or major organ tissue degeneration. In contrast, anti-tumor activity was completely abolished in EC0305 mice that were co-dosed with an excess of a non-toxic folate-containing analog, thereby confirming that this agent's anti-tumor effect was mediated by FRs. The advantage provided by folate conjugation was further proven by the un-targeted free drug, which was found to be completely inactive at both tolerable and highly toxic dose levels.

Conclusions: These results collectively show that a potent anti-proliferative tubulysin compound can be specifically delivered to FR-positive tumors to provide substantial therapeutic benefit using well-tolerable dosing regimens.
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Effects of Central Penicillin Administration on Neuronal Response of the Nucleus Reticularis Gigantocellularis

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Background: Reticular neurons of medulla oblongata participate in a large variety of sensory, motor and autonomic functions. The gigantocellular reticular nucleus is a part of reticular neuronal network with a high density of GABAergic neurons. This structure plays the regulatory, integrative and coordinative role in cardiovascular and respiratory control. The aim of this paper was to study the neuronal responses of bulbar reticular units after disturbance of inhibitory processes.

Methods: Penicillin was used as a convulsant agent, which is able to non-selectively block GABAmediated synaptic inhibition. Benzyl penicillin (50 U/µl) was locally applied by pressure microinjection into the central part of reticular gigantocellular nucleus of 65 anesthetized or paralyzed Wistar rats. Effects of penicillin administration were examined on the firing rate, discharge pattern and interspike intervals of reticular units.

Results: Penicillin caused enhancement of the number of active firing neurons (138%) and reorganization the spatial neuronal architecture of the examined structure. In gigantocellular nucleus penicillin application induced excitory responses in 78% of extracellularly recorded units and inhibition in 15% of them. 18% of recorded neurons exhibited high-frequency firing activity. These facts support data which showed that this reticular region includes functionally labile cells capable of transforming tonic activity into burst pattern. The peak and mode of interspike intervals histograms were shifted toward shorter intervals and their amplitude was increased after penicillin microinjection. Distributions of interspike intervals histograms were transformed (unimodal to exponential, multimodal to unimodal).

In no case did vehicle administration produce the significant changes in neuronal activity.

Conclusion: We concluded that neurons of reticular gigantocellular nucleus are very sensitive to the local blockage of synaptic inhibition by penicillin within of medulla oblongata. Results suggest a considerable physiological role of medullary inhibitory mechanisms in homeostasis maintenance.

Fentanyl: How Delivery System Can Modify Clinical Properties Of Molecule

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Fentanyl (N-(phenyl-N-(1-phenethyl-4-piperidinyl)propanamide) was first synthesized by Paul Janssen in 1959. Fentanyl was introduced into medical practice in the 1960s. It has long been established in anesthetics and ITU practice due to potent analgesic action, intravenous administration, very rapid onset, short half-life and low incidence of histamine release. Fentanyl administrated orally has low bioavailability due to first pass metabolism. Intravenous route of administration have limited significantly fentanyl application outside anesthesia and ITU until ’magic bullets’ was applied. Fentanyl ‘magic bullets’ have form of different delivery systems, which significantly modify clinical properties of molecule.

Development of transdermal fentanyl patches allows avoiding intravenous route of administration offers very long opioid analgesia (up to 72 hours) and could be used for patients with swallowing difficulties. Transdermal fentanyl patches are widely used for background pain. Utilization of transcutaneous route of administration creates very fast rescue medication for breakthrough pain. Transbuccal preparation is available as lozenges and effervescent tablet. Electrophoretic fentanyl patch offers postoperative patient controlled analgesia system, which eliminates need of syringe driver and intravenous (or subcutaneous) contact. Fentanyl is administrated on patient request (pressing button) by transdermal electrophoresis. Application of ’magic bullets’ (delivery systems) transfomed simple molecule into number of different systems, which could be used widely in cancer and non-cancer pain management.

COKKEN: A New Strategy For Predicting The Chemosensitivity Of Human Cancers And Its Application To Drug Discovery

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The U.S. National Cancer Institute has used a panel of 60 diverse human cancer cell lines (the NCI-60) to screen >100,000 chemical compounds for anticancer activity. However, not all important cancer types are included on the panel nor are drug responses on the panel predictive of clinical efficacy in patients. We thus asked whether it would be possible to identify common chemosensitivity biomarkers from that rich database to predict drug activity in cell lines not included in the NCI-60 panel or, even further, clinical responses in patients with tumors. We address that challenge by developing a novel pharmacogenomic approach “Co-expression Extrapolator6” (COKKEN), which can effectively identify concordant genomic chemosensitivity biomarkers between two independent expression profiling data sets, here extrapolating the genomic expression patterns of NCI-60 biomarkers with those of clinical tumors. Applying our COKKEN approach in a prospective fashion, we predicted anticancer drug activities on completely independent bladder cancer, which is not included in the NCI-60 panel, and chemotherapeutic responses and survival of breast, bladder, and ovarian cancer patients treated with commonly used single and multi-agent chemotherapies. We also used COKKEN for in silico screening of 45,545 compounds and identify a novel agent with superior growth inhibition activity against human bladder cancer.
Erythropoietin-Binding Protein And Its Antibodies For Possible Clinical Application

Systematic Discovery Of Novel Multi-Target Therapeutics: Finding The New Magic Bullets

**Background:** Hypertension (H), prevalent worldwide, is associated with high morbidity and mortality. Many investigators believe that genetic components are important in the etiology of H. In embryo transfer studies, genetic factors dominantly affected blood pressure (BP) (IP < 0.0001) and its fluctuations (IP < 0.032), when we transformed one-cell homzygous embryos into hypertensive or normotensive rats’ oviduct, and pups were cross-suckled at birth. Erythropoietin (Epo) induced H is the most serious complication in Epo treatments (Rx). Uncontrollable BP rise in 1/3 of patients, organ-damage and even death have resulted in the U.S. Food and Drug Administration “re-evaluating” the safety of Epo.

**Method & Result:** Human Epo-binding protein (Epo-bp) and anti-Epo-bp antibodies (a-Epo-bp) were genetically engineered to test the variability of EpoRx. Epo Rx increased hematocrit (Ht) markedly overall when compared to saline (S), Epo Rx, and Epo-bp-Rx (Rx 0.62 ± 0.43, 0.44, and 0.44, respectively) and at each of the 6 test times (all P < 0.0001). They had almost no effect on the Epo-induced Ht increase. Cyclic BPs for Epo Rx, S, Epo-bp, and Epo-bp-Rx were 126 ± 2 vs. 116 ± 2, 118 ± 2 and 117 ± 2 mm Hg, respectively (each P < 0.0001). Spleenomelymphatized each rat in the Epo Rx: in grams 1.58 ± Epo Rx vs. 0.86 in S, 0.89 in Epo-bp, and 0.85 in Epo-bp-Rx (each P < 0.0001). Ligation-binding sites were detected using fluorescein-labeled Epo-bp & Epo-bp in various blood reactors. We developed dual-specific kits to detect Epo, Epo-bp and their antibodies to differentiate Epo- from EpoR-related diseases. Epo levels in serum & plasma: 25 ± 4 vs. 20 ± 2; Epo Rx: 24 ± 2 ± 25 ± 1 mlU/ml, respectively. Cell membrane proteins play a key role in cell-cell communication. Thus, exploratory membrane protein polymorphisms and hormonal interactions may expand our knowledge of the normal and abnormal physiological process, and lead to the development of a new strategy in those Rx.

**Conclusion:** Epo-bp and u-Epo-bp effectively eliminate Epo-induced H without affecting Ht. They are predicted to be therapeutic agents for hematopoietic malignancy, and used as diagnostic tools at test sites and quick detection of the athletistic abuse of Epo as a doping agent, and as research tools, not only for EpoRx, but also for many other circulatory, vessel and tissue-related malignancies.

Clinical implications of our materials are enormous and diverse, and provide hope for patients suffering from those problems without damaging adversity, the perfect concept for the Magic Bullet envisioned by Dr. Ehrlich.

**LEMAIRE S, VAN BABEMBE F TULKENS PM**

Université Catholique de Louvain, Brussels, Belgium.

**Background:** Early studies showed that MRSA strains become susceptible to -lactams when they are exposed to acidic pH (pH ≤ 5.5) (Sabath et al., AAC, 1972). Because S. aureus survives in the phagolysosomes of macrophages (where the pH is in the acidic range), we have examined the intracellular susceptibility of MRSA strains to claxocillin and meropenem.

**Methods and conclusions:** We have discovered multiple unexpected synergistic combinations in oncology indications. In one example, the combination of an anti-parasitic agent, pantamidine and a phenothiazine anti-psychotic, chlorpromazine exerts an anti-proliferative effect through synergistic action on the mitotic targets KSP and PRL phosphatases synergize in mitosis to inhibit tumor cell growth. In a second example, a multi-target mechanism screen in multiple myeloma cell lines has revealed novel and highly selective synergistic interactions between molecular pathways not previously known in multiple myeloma.

**Conclusions:** The systematic survey of multi-target mechanisms enables the discovery of interesting new biology, the definition of specific target pairs for therapeutic development and demonstrates the power of combination biology for drug discovery.

**Novel synergistic combination therapies are validated in secondary disease relevant in vitro and in vivo model systems and rapidly advanced to clinical proof of concept studies.**

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**Magic Activity Of Beta-Lactam Antibiotics Against Intracellular Methicillin-Resistant S. Aureus (MRSA): Role Of Acidic pH**

**LEMAIRE S, VAN BABEMBE F TULKENS PM**

Université Catholique de Louvain, Brussels, Belgium.

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Molecular Targeting of the bcl-2 Oncogene for Staging and Therapy of B-Cell Lymphoma

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Background: The 8-70 lymphoma/follicular-2 (bcl-2) oncogene is a dominant inhibitor of apoptosis, correlating with resistance to radiation and chemotherapy, high tumor burden, and a poor survival in non-Hodgkin’s B-cell lymphoma (NHL). NHL also expresses type 2 somatostatin receptors (SSTR2) in 87% of cases, making it attractive for delivery of intracellular tumor-targeting agents.

Methods: A bcl-2 antisense peptide nucleic acid (PNA) conjugated to a SSTR2-targeting peptide, anti-bcl-2-Tyr-177-cteolate, was evaluated for its in gamma scintigraphy and single photon emission computed tomography (SPECT). Cu-potassium emission tomography (PET) and 111Lu targeted radiotherapy (TRT) in NHL cells in culture, mouse models of human NHL, and dogs with spontaneously occurring canine NHL-positive Mec-1 cells (n = 3) or bcl-2-mRNA-negative Ramos (n = 3) xenografts were used for in vivo microSPECT or in vivo microPET imaging. The 111Lu conjugate was also used for gamma scintigraphy of canine NHL patients (n = 15). The 111Lu conjugate was evaluated in vitro for TRT in Mec-1 cells (n = 3).

Results: Incubation of Mec-1 cells with anti-bcl-2-Tyr-177-cteolate showed a 51% decrease in bcl-2 protein synthesis, suggesting that the target mRNA function had been perturbed by a specific antisense effect. Both 111In microSPECT and 111Cu microPET could detect Mec-1 tumors, but not Ramos tumors, in SCID mice (p < 0.05). Gamma scintigraphy demonstrated the utility of the 111In conjugate for molecular imaging of the bcl-2 oncogene in canine NHL. In vitro Mec-1 cell studies showed that the 111In conjugate had at least an additive effect on cell viability, compared to controls for targeted radioactivity and bcl-2 antisense activity.

Conclusions: Imaging studies demonstrated that: 1) 111In- and 111Cu-anti-bcl-2-Tyr-177-cteolate were specific for bcl-2 mRNA-positive canine NHL xenografts. 2) Imaging of canine NHL established bcl-2 expression as a clinical and molecular model relevant to human disease. 3) TRT studies of the 111Lu conjugate in Mec-1 cells demonstrated down-regulation of bcl-2 with radiation insult, creating a NHL therapy agent acting through two targeted anti-tumor mechanisms.

Development of Novel γ-Conopeptide Inhibitors of the Norepinephrine Transporter for the Treatment of Severe Pain

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Background: Signal transduction therapy was introduced into cancer therapeutics in the early 1990s after the first tyrosinokinases and anti-Her2 antibodies were developed. Currently there are a few antibodies like Herceptin, Avastin and Eribulix in the clinic as well as eight tyrosine phosphorylation inhibitors.

Methods: The design and synthesis of novel protein kinase inhibitors that are ATP non-competitive inhibitors will be presented. We shall demonstrate the successful use of a combination of an EGFR kinase inhibitor with our novel PKB/Akt inhibitor and a DNA damaging agent to strongly inhibit glioblastoma, which overexpresses EGFR and lacks PTEN. We shall also present the design, synthesis and performance of a chemical vector homing for the EGFR receptor. The vector is loaded with Polycycl and due to the ability of the EGFR to internalize the vector; Polycycl is inserted into the cell, eliciting a strong anti-tumor response. Here we shall specifically discuss an EGFR homing vector carrying Polycycl.

Results: In view of the success of targeted cancer therapy since the early studies on tyrosinokinases (1988) we have enhanced our efforts to improve the performance of targeted therapies by following new paradigms. In this talk I will present three ongoing studies in our laboratory (1) the development of novel substrate competitive PKB/Akt inhibitors and how they perform in the treatment of prostate cancer and brain cancer in nude mice. (2) The development of novel allosteric IGFR inhibitors that lead to the degradation of the IRS proteins and their highly potent in vivo efficacy against small cell disseminated cancer, breast cancer, and prostate cancer in nude mice and (3) the eradication of EGFR over-expressing glioblastoma xenografts in mice by using a targeted chemotherapy (EGFR(PolyCycl)) approach is highly effective due to the targeted bystander effect induced by the Polycycl that is inserted selectively into the EGFR over-expressing tumor cells. This is in part by far the most effective signal transduction therapy regimen in the treatment of EGFR over-expressing tumors, in experimental animals.

Conclusions: (1) Non-ATP competitive protein kinase inhibitors are highly efficient agents and may be used with success to treat various cancers. (2) A chemical vector homing to a receptor, which is over-expressed in tumors and which can be targeted in situ can induce complete eradication of the tumor if loaded with Polycycl. This approach can be in principle utilized with many receptors as a target, where the example given here is an EGFR homing vector.

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Background: Bacterial resistance to an antibiotic may result from survival in a suddenly strong antibiotic or in sub-minimum inhibitory concentration of the drugs. Their shared proteins responsible for the resistance should be potential targets for designing of new drugs to inhibit the growth of the antibiotic-resistant bacteria. Aims: 1) To identify sharedly altered outer membrane proteins (OM proteins) that are responsible for chloramphenicol-resistant (CAP)-resistant Escherichia coli and for survival in medium with suddenly strong CAP treatment. 2) To develop a novel method of specific antibody combating bacterial growth based on these shared OM proteins.

Methods: Comparative sub-proteinetic methodologies were used to identify differentially expressed OM proteins from E. coli responding to suddenly increased CAP treatment and from CAP-resistant E. coli selected from survivors after ten passages of subculture with the sub-inhibitory concentration of CAP. After the shared OM protein responsible for CAP between the two ways of the exposures to the antibiotic treatment were determined, their capacity in antibiotic resistance was further investigated by their mutants. A specific antibody showing growth effect was developed to inhibit the activity of CAP-resistant OM proteins. The incoluates of CAP-R, CAP-R, OmpC, OmpT and OmpW were separately cultivated in 5 mL fresh LB medium at 37°C overnight, and then the cultures were diluted 1:100 into 5 mL fresh LB medium to obtain the desired cell density (OD600nm=0.5, 106 CFU/ml). Pellet from 200 µl of each of six cultures was obtained by centrifugation at 9000×g for 4 min and was separately incubated with 50 µl of rabbit pre-immune serum and immune serum against ToIC, OmpC, OmpT or OmpW at 37°C for 1 h. After washing, the cultures were separately cultivated in 200 µl fresh LB medium and then were dialyzed 1:1000 into 5 mL LB medium without or with 1/8 µl CAP. The growth of these cultures were incubated at 37°C for 9 h and it was measured at OD600nm for survival capacity.

Results: Six differential OM proteins and an unknown location protein were determined to be sharedly CAP-resistant-related proteins with the use of 2-DE/MS, Western blotting and gene mutant methods, in which ToIC, OmpC, OmpT and OmpW were critical agents for the antibiotic resistance. Furthermore, only anti-ToIC showed a significant inhibition on bacterial growth in medium with CAP when antisera to TolC, OmpC, OmpT and OmpW were separately applied. The growth of CAP-resistant E. coli and its original strain was completely inhibited when they were bound with anti-ToIC and survived in 1/8 µl of CAP, which was equal to the activity of ToIC when it did in the same conditions of the antibiotic combination.

Conclusions: 1) Bacterial growth can be combated using the antibody specific to ToIC, suggesting a novel insight into the selection of antibiotic-resistant bacteria. Combination therapy involving antibiotics that enhance the expression of an antibiotic target could be other drug alone, which gives a novel insight into battling infection by antibiotic-resistant bacteria. This work was sponsored by grants from NSFC project 30530610, Guangzhou Key Project 2006Z3-E0251 and Guangdong NSF key project (7117845).

Antibody-Therapeutic Targeting Of Tolic For Growth-Combating Of Antibiotic-Resistant Escherichia Coli

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Influenza viruses, both seasonal and pandemic, pose an ongoing threat to global health. Vaccines remain the best means of controlling influenza infection and spread, but the current worldwide capacity for seasonal influenza vaccine production falls far short of the capacity needed to provide sufficient vaccine for mass immunization in the event of a pandemic outbreak. To increase the supply of seasonal vaccine and the surge capacity for producing pandemic vaccine, the U.S. Government has established programs to facilitate the development of new technologies for enhancing the domestic and international influenza vaccine manufacturing infrastructure. Three major initiatives have been launched to support advanced development of: i) cell culture-based influenza vaccines; ii) novel dose-sparing adjunct technologies for pandemic influenza vaccines; and iii) recombinant influenza vaccines. Funding has been provided under these programs for process development, clinical studies, establishment of manufacturing facilities, and other activities leading to licensure of the vaccines by U.S. Food and Drug Administration. The U.S. Government has awarded six contracts for the development of cell-based influenza vaccines. The cell-based technologies will complement the currently licensed egg-based manufacturing technology to meet the USG’s stated goal of producing 450 million doses of vaccines within six months. Three contracts have been awarded for the development of novel adjuvants that would allow antigen-sparing in H5N1 vaccines. As the non-adjuvanted H5N1 vaccine is poorly immunogenic, and thus requires high doses of antigen to be effective in humans, development of these adjuvants may be critical for meeting pandemic vaccine needs. The successful conclusion of these programs will fundamentally alter the U.S. and global influenza vaccine manufacturing base and provide increased vaccine production capacity for pandemic preparedness.

Pharmacogenomic Targeting Of Ehrlich's Magic Bullet

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Background: Paul Ehrlich focused on targeting microbial invaders and renegade (cancer) cells. Despite his valiant efforts, both the enemies within, and the invaders have proved resilient. However, considerable progress has been made since and we demonstrate the wide applicability of his vision and approach.

Methods: We surveyed drugs in current and investigational use, and highlight the dramatic progress made through application of Ehrlichian principles of drug discovery and optimization.

Results: Ehrlich's vision, was visual in his approach. His genius was to recognize that selective staining of microbes and cancer cells highlighted a route to drug selectivity. We still use the same approach but probe at a deeper genomic level, and on a vastly wider scale using gene expression microarrays. Ehrlich used both small molecules and macromolecular agents in his armamentarium. We maintain this to probe an array of selective small molecules for targeting specific enzymes (e.g. matrikin) and disease pathways, and highly selective macromolecular drugs (e.g. monoclonal antibodies) for virtually every degenerative disease. Moreover, we now identify the enemy with much greater precision (e.g. fine molecular classification of clinically similar diseases, and identification of resistance genes and epitopes to develop effective vaccines against enemies, both seen and unseen). Use of combinations of magic bullets causes less collateral damage (e.g. infections, rheumatoid arthritis), and lowers the risk of resistance. Unraveling more of the mysteries of our DNA self, helps us develop new magic bullets (e.g. siRNA), and new shuttles for our wounded troops (e.g. gene vectors) and sometimess act as decoys against recalcitrant (e.g. suicide gene therapy). Learning how to navigate in and out of tunnels (transmembrane transporter pharmacogenomics) ensures that our magic bullets are aimed more precisely. We illustrate these visually in our presentation.

Conclusions: Ehrlich's magic bullet has undergone considerable development. With our ageing populations, Ehrlich's disciples have to contend with an increasing number of enemies from within as well as the persistently challenging invaders. It is of course obvious that the enemy will always win in the end but new Ehrlichian weaponry is allowing us to prolong life and improve its quality.

Enhancement Of Radiation Or Chemotherapeutic Effects Of Para-Aminobenzoic Acid (PABA) And A Novel Analog On Melanoma: Preclinical Studies And Phase I Studies

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Background: B16F10 melanoma cells cultured in media containing PABA resulted in a depigmentation and inhibition of tyrosinase activity relative to cells grown in PABA deficient media. We have determined that PABA's potentiation of radiation or paclitaxel cytotoxicity, mechanistic studies showed inhibition of cell cycle arrest by up-regulation of CDC25A and down-regulation of p21WAF1 and BRCA2. A Phase I study of combination PABA, Paclitaxel (P) and carboplatin(IC) in metastatic melanoma patients who had failed at least one prior chemotherapy regimen was initiated. Structure-activity studies utilizing tyrosinase activity identified C45, as a more potent PABA analog.

Methods: Cell culture and murine models assessed the effects of PABA and C45 on melanoma tumor growth and gene expression. Cell cycle modulation and potentiation of cytotoxicity was greatest for P. We then enrolled patients (pts) with metastatic melanoma who had failed at least one prior chemotherapy regimen to receive PABA in combination with C and dose-escalated P. Measurement of plasma pharmacokinetics (PK) of PABA prior to, and 24 hours (h) after carboplatin/paclitaxel were obtained as well as PK of P after PABA administration. Results: C45 showed modulation of cellular proliferation in Lewis, Lung and B16F10 melanoma. It potentiated the in vivo activity of P. Temozolomide and Alimta and external beam radiation in M21 and B16F10 models. In the phase I, 19 pts were enrolled with 18 available for assessment. One grade IV neutropenia was seen and no dose-limiting toxicities even at doses of C AUC 5, and P 175 mgm2/that are in common use. PABA PK for dose levels IV showed a mean Cmax of 14 µg/ml ± 7.9 µg/ml at 0.5 h, and a mean elimination half-life of 2 h. In the presence of P, the mean half-life of PABA was 4.6 h. Four partial responses were seen and 2 had stable disease with duration of response ranging from 12-32 weeks.

Conclusions: PABA is safely administered in combination with full dose P and C in pts with refractory metastatic melanoma. Our enhanced therapeutic activity with low toxicity, justifies a phase II efficacy trial. Studies are continuing with the evaluation of the dose response and pharmacokinetics of the PABA analog, C45.

Authors' disclosure statement (not counting towards the character count): Supported in part by grants from NIH CA 91645, 2P3O CA16087-28, The Chemotherapy Foundation, and from Cancer Innovations, Inc.
Advantages of Multiple Drug Interactions: Combinatorial Treatments Using Neural Networks

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Background: There are few Magic Bullets since drugs designed for one specific effect also interact with many other molecules in a cell. Instead of a disadvantage, we can turn this network of interactions into an advantage. If we can infer enough information about this network, from a limited set of experiments, we can predict which combinations of drugs will interact with each other, in just the right way, to have the most targeted effect with the fewest side effects. We call this approach Combinatorial Multi-Component Therapy (CMCT).

Methods: To illustrate this approach we constructed model test networks of linear or non-linear drug interactions. We then trained an artificial neural network (ANN) on a limited data set of drug inputs presented one-at-a-time and pairs-at-a-time and their output effect predicted by these models. We optimized the performance of the ANN by using only one output, softening the transfer function between the input and hidden layers, using four times as many units in the hidden layers as the input, and taking the logarithm of the output values.

Results: For the model test networks, the ANN, trained on only very limited data, accurately computed the output effect for different combinations of input drugs. For example, 99% of the outputs had errors of less than 10% for all 32,768 combinations of 15 inputs that are either (0,1) in a highly nonlinear model of drug interactions.

Conclusions: These test results suggest that this CMCT approach may be of value in determining how combinations of drugs can be effectively used to achieve specific therapeutic results.

Millions of young people consume “Ecstasy” (MDMA), indicating that MDMA has strong rewarding effects. MDMA induces a state of well-being, elevated mood, moderate psychomotor stimulation, few perceptual changes and little anxiety.

MDMA acts at the presynaptic monoamine transporter and releases serotonin, dopamine, and norepinephrine by reversing the transport of these monoamines. While the neurochemical effects of MDMA have been well described in preclinical models, it is less clear how the neurochemistry translates into to the psychotrophic effects in humans. The rewarding effects of drugs of abuse are commonly attributed to the release of dopamine in the mesolimbic system. Indeed, pharmacological blockade of dopaminergic D2 receptors with haloperidol attenuated the positive mood effects of MDMA and produced increased adverse effects including anxiety. Serotonin uptake inhibitors (SSRIs) decrease MDMA-induced serotonin release by blocking the interaction of MDMA with the serotonin uptake site. In humans, administration of an SSRI prior to MDMA markedly decreased all subjective and physiological effects of MDMA including its positive effects, reduced adverse effects, the subjective changes and the increase in blood pressure and heart rate. Further, blockade of the postsynaptic serotinergic 5-HT7 receptors selectively attenuated MDMA-induced perceptual changes. Together these results indicate that the MDMA-induced effects are overall due to release of endogenous serotonin with contributing effects of dopamine release to positive mood. MDMA-induced hallucinogen-like perceptual changes can be linked to 5-HT7 receptor stimulation.

Ecstasy use is associated with serious adverse effects including hyperthermia, liver failure, hypertensive brain edema and cardiovascular complications. Furthermore, there are concerns that heavy Ecstasy use may lead to lasting cognitive impairment due to serotonergic neurotoxicity.

So far, the magic Ecstasy pills induce short-term happiness but severe adverse effects and potentially persisting cognitive effects are a high price to pay. Recent rodent studies indicate that the Alzheimer treatment memantine prevented MDMA-induced neurotoxicity and cognitive deficits. The question is open whether the magic bullet – the safe pill for happiness – will contain a psychostimulant and a neuroprotective treatment for Alzheimer’s disease.

Ecstasy – The Pharmacology of Happiness

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Peripherally Administered TrkB Agonists Cause AppetiteEnhancement and Weight Gain in Non-Human Primates

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Background: Brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT4), ligands of receptor tyrosine kinase TrkB, are anorexogenic when administered peripherally or centrally in mice. We hypothesized that like rodents, peripheral or central administration of TrkB agonists could reduce body weight and food intake in non-human primate species. If true, TrkB agonism may be a therapeutic approach for human obesity.

Methods: 2 rhesus monkeys received a dose escalation of BDNF (n=4) or NT4 (n=4) by intracerebroventricular (ICV) delivery. 24 cymolugous macaques were administered with NT4 either daily intravenously (IV), or daily subcutaneously (SC), or with a TrkB agonist antibody twice weekly IV. 6 obese baboons were given NT4 (n=3) or vehicle (n=3) daily IV. These baboons were later administered NT4 (n=3) or vehicle (n=3) IV twice weekly.

Results: ICV administration of NT4 or BDNF into rhesus monkeys resulted in a dose-dependent suppression of food intake. Daily ICV dosing of 2mg/kg of NT4, or IV dosing of 2mg/kg in cynos monkeys resulted in a 2 to 3-fold increase in daily food intake, and a 1.6 to 2.3-fold increase in cumulative food intake respectively. Daily SC or IV injections resulted in a 16% (SC for 21 days, p<0.001) or 33% (IV for 30days, p<0.001) increase of body weight respectively. Twice a week IV dosing (21 days) of the TrkB agonist antibody (5mg/kg) in cynos monkeys resulted in a 40% increase in cumulative food intake (p<0.001) and a 10% increase in body weight (p<0.01). Obese baboons given daily IV injections of NT4 (2mg/kg) increased their daily food intake by 2 to 3-fold, and their cumulative food intake (25 days) by 2.5 fold. Body weight increased by 16% (p<0.01).

Conclusions: 1) We observed a novel orexigenic response to peripheral administration of TrkB agonists, that is contrary to the anorexogenic response of peripheral or central TrkB agonism in mice. 2) Peripheral administration of NT4 was well-tolerated, suggesting that TrkB agonism could be a feasible therapeutic for anorexia or cachexia.
Hyaluronan-Mediated Transformation And Relapse Of Prostate Cancer

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INTRODUCTION: Interaction between extracellular matrices (ECM) and cancer cell receptors frequently alters signal transduction pathways, which lead to malignant transformation and metastasis. Hyaluronan (HA), an ECM tumor promoter and enhancer, is synthesized by stromal fibroblasts in response to paracrine factors produced by tumor cells. This type of tumor-stromal interaction plays a crucial role in the stimulation and promotion of cancer cell tumorigenesis. However, the molecular mechanism remains unclear.

MATERIALS & METHODS: Human microrna analysis and immunodetection in human CaP tissue arrays were used to screen differentially expressed oncomirs markers from patients with various stages of prostate cancer (CaP). In vitro tumorigenecity assays were performed to evaluate the loss-of-oncogene function, including cell-cycle flow cytometry (cell proliferation), cell invasion chamber (migration and invasion), and adhesion to a human bone marrow endothelial cell (hBMEC) monolayer (metastasis).

RESULTS: We observed that HA-mediated CD168++, a receptor for HA-mediated motility (RHAMM), and its downstream signal molecules, including ROCK1, Gab-1, PI3Kγ110s and eIF4E, promote the malignant progression of hormone-refractory CaP (Carcinogenesis 28: 310-320, 2007). In normal prostate, androgen receptor (AR) serves as a tumor suppressor against the HA-stimulated CD168++ signaling by binding to the AR-binding domain (ABD) that bind and hydrolyze ATP. HA is also found to regulate the transcription of CD168 mRNA in the presence of androgen. The results of in-vitro tumorigenecity assays further showed that CaP cells with deficiency or mutation of AR signaling increase the malignancy of cancer tumorigenecity in terms of cell proliferation, cell invasion and metastasis into the hBMEC monolayer. The expression of mir-146 against the key kinase ROCK1 of the HA–CD168++ signaling pathway can reverse the HA-stimulated malignancy (RNAi 14: 417-424, 2008).

CONCLUSION: HA activates the signal transduction cascade of CD168++–ROCK1–PI3Kγ110–eIF4E, which can be prevented by ROCK depletion using mir-146. Therefore, our study suggests that the combination of current cancer therapy with an anti-ROCK agent may lead to beneficial results in preventing HA-mediated cancer transformation and relapse.

 dodging the bullets: an update on the multi-drug efflux pump P-glycoprotein

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Background: Human P-glycoprotein (Pgp, ABCB1) is a clinically-relevant drug exporter of the ATP Binding Cassette transporter family. The protein has two transmembrane domains (TMD) that contain the drug binding sites, and two nucleotide binding domains (NBD) that bind and hydrolyze ATP to drive the drug transport cycle. Structural studies on bacterial homologues indicate that the two NBDs adopt one of two composite sites of highly conserved motifs that catalyze ATP hydrolysis. Each site comprises a Walker A and B motif, stacking aromatic and γ-phosphate of ATP via a water molecule. The Q-loop links the two Walker domains of the NBD, and also interdigitates with the TMDs.

Methods: We systematically examined the importance of these motifs in the function of Pgp using a 1:2:1:1 multipurpose purification of Pgp was measured by colorimetric assay.

Results: Single mutations introduced into the Walker A or B motifs render the protein virtually inactive. However, for the stacking aromatic, the ABC signature and the D-, H- and Q-loop motifs, mutation of one NBD has no, or minimal effect, on drug transport, but mutation of the motif in both NBDs has a strong, synergistic, negative effect. The high level of activity in the single Q-loop mutants was particularly surprising as data from mouse Pgp published previously suggests that these should be severely debilitating. However, on purification, the single Q-loop mutants of human Pgp exhibit ~10% of the drug-stimulated ATPase activity of wild type Pgp and the double mutant is inactive.

Conclusions: The emerging picture is that canonical Walker A and B motifs are essential for drug transport, however, there is redundancy in the mechanism for the stacking aromatic, the ABC signature and the D-, H- and Q-loop motifs, in the absence of either Q-loop glutamate. Pgp appears to be flexible to loose to tolerate defects. Together with structural data, this suggests that the Q-loop is the fulcrum of the molecular mechanism and an important conduit for energy transduction between domains.

Authors’ disclosure statement: The abstract describes unpublished data

Verotoxin (Shiga toxin) binding to its receptor glycolipid, globotriaosylceramide, provides a new antineoplastic tool and physiologically-based approaches to tumour cell drug resistance

VEROTOXIN (SHIGA TOXIN) BINDING TO ITS RECEPTOR GLYCOLIPID, GLOBOTRIAOSYL CERAMIDE, PROVIDES A NEW ANTI-NEOPLASTIC TOOL AND PHYSIOLOGICALLY-BASED APPROACHES TO TUMOUR CELL DRUG RESISTANCE

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Background Verotoxin 1 (Shiga toxin) kills cells expressing its receptor glyco phospholipid/GSL, globotriaosyl ceramide (Gb3) expressed in a range of targets. Gb3 is upregulated in many human cancers including breast, ovarian, colon, glioblastoma, meningioma, renal, testicular. Although Gb3 is expressed on a few normal tissues these are not targeted in a primate model, suggesting that Gb3 may not be in DRMs in such cases. Intratumoral VT1 injection reduces growth rate and eliminates human GB3 positive tumors xenografts (astrocytoma, meningioma, colon and renal carcinomas) grown in mice. Gb3 is also expressed in human neovascularisation, indicating that VT1 has antineoplastic and antiangiogenic activity. Primate studies established a safe VT1 dosage for clinical trials.

Methods: MDR1 transfected MDCK cells were used to assess MDR1 inhibition and link between MDR1 processing and GSL biosynthesis and polarized C2BBe1 gastrointestinal epithelial cells to use measure MDR1 mediated drug fluxes. Results: Gb3 is particularly elevated in MDR1 expressing drug resistant tumour xenografts which led us to determine that MDR1 is a Gb3 GSL Biparase, involved in neutral GSL biosynthesis. Inhibition of GSL biosynthesis prevents cell surface MDR1 expression in cell lines from drug resistant tumours, though intracellular MDR1 accumulates. Cell surface MDR1 showed significant colocalization with Gb3 as monitored by FTET labeling. MDR1 is found within DRMs and treatment of drug resistant cells with Verotoxin reversed this effect. Automated image content analysis was used to quantify DRM domains. Gb3 was found to bind to DRM domains, and proved the first physiologically-based inhibitor of MDR1. MDR1 mediated rhodamine efflux was prevented and adamantrylGb3 reversed cell resistance to vinblastin. Gastrointestinal MDR1 also reduces oral drug bioavailability. MDR1-mediated digoxin and vinblastin efflux in human intestinal epithelial cells was prevented by adamanylGb3.

Conclusion: Gb3 provides an antineoplastic target and new insight into tumour drug resistance and drug oral bioavailability.


Fluorescent Biosensors to Detect Magic Bullets against Multi-Drug Resistant Bacteria

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Background: Rising antimicrobial resistance of major pathogens limits therapeutic options. Identifying novel antimicrobial targets through genome-derived, target-based approaches have widely failed in the past decades. Whole-cell bacterial biosensors with promotor-inducible fluorescent reporters provide an attractive alternative to target-based approaches. They are compatible for high-throughput-screening with a LSR 2 flow cytometer with a high-throughput sampler. The system measures 13 parameters per well. Automated image content analysis records a population-based dataset suitable for drug discovery. Several fluorescent biosensors that report specific disturbance in cell wall and protein biosynthesis pathways were found. An optimal assay protocol for screening in a 96-well microtiter plate format was developed.

Methods: Whole-cell bacterial biosensors and their promoter-inducible fluorescent reporters provide an attractive alternative. They are compatible for high-throughput-screening with a LSR 2 flow cytometer with a high-throughput sampler. The system measures 13 parameters per well. Automated image content analysis records a population-based dataset suitable for drug discovery. Several fluorescent biosensors that report specific disturbance in cell wall and protein biosynthesis pathways were found. An optimal assay protocol for screening in a 96-well microtiter plate format was developed.

Results: These biosensors read only to inhibitors that reach significant concentrations in live bacteria and not to unspecific stressors. We optimized these biosensors for HTS screening in a 96-well plate format.

In comparison to luciferase or beta-galactosidase based biosensors, we can combine in a single well up to six fluorescent biosensors expressing different variants of GFP and simultaneously detect distinctive responses of each individual biosensor in the composite using automated multi-color FACs analysis. Our biosensors are more sensitive than grown assays and highly specific for well-defined targets. They are compatible for high-throughput-screening with a LSR2 flow cytometer with a high throughput sampler. The system measures 13 parameters per well. Automated image content analysis records a population-based dataset suitable for drug discovery. Cell wall permeability and fluorescent protein expression in up to six biosensors can be measured with compounds.

Conclusions: A new sophisticated screening system was developed. The system can detect specific groups of antimicrobial compounds. Incubation times are short and only low compound concentrations are needed. This opens up new perspectives for antimicrobial screening.

Abstracts
Trans-lymphatic Chemotherapy

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Background: Lymph node metastasis is a significant prognostic factor for most cancers. Failure to control lymphatic metastases may result in local recurrence and systemic metastasis. A trans-lymphatic chemotherapy technology was developed to control lymphatic metastases.

Methods: Trans-lymphatic technology exploits an implantable gelatin sponge impregnated with biodegradable polymer microparticulate anticancer agent and is referred to as microparticulate lymphatic targeting system (MLTS). When the system is formulated for paclitaxel (PTX) or doxorubicin (Dox), it is designated as MLTS-PTX or MLTS-Dox respectively. The systems were characterized in vitro. The pharmacokinetics (PK) of MLTS-PTX was studied in rats with comparison to injectable PTX given i.v. or intraperitoneally (i.p). The therapeutic efficacy was examined in an orthotopic lung cancer model. MLTS-PTX was placed into the pleural cavity when the tumor lung was resected 14 days after orthotopic tumor implantation. Tumor recurrences were assessed 32 days following the procedure. The therapeutic efficacy of MLTS-Dox was examined in SCID mice bearing DLD1 orthotopic colon cancer model. MLTS-PTX was placed into the pleural cavity when the tumor lung was resected 14 days after orthotopic tumor implantation. Tumor recurrences were assessed 32 days following the procedure.

Results: Both systems exhibit controlled drug release properties in vitro. The microspheres were selectively taken up by the lymphatics and delivered to the regional lymph nodes as the sponge disintegrated. PK studies revealed a significantly higher AUC in mediastinal lymph nodes with i.pl placement of MLTS-PTX as compared to i.v or i.p administration of PTX. This represents approximately a 400-fold increase in lymphatic drug exposure as compared to i.v dosing. Peak plasma concentration was significantly reduced. There was an 80% reduction in lymph node metastasis with MLTS-PTX treatment. The microparticulate PTX was microscopically evident in the targeted lymph nodes. Similarly, MLTS-Dox significantly decreased the incidence of lymph node metastasis in the treatment arm (20%) as compared to the controls (100%). Microparticulate Dox were seen in the targeted lymph nodes.

Conclusions: Trans-lymphatic targeted chemotherapy reduces lymph node metastasis in both lung cancer and colon cancer models. This effect may be attributed to the improved lymphatic distribution of the therapeutic agents.

An enzymatic approach for developing heparan sulfate-based drugs

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Background: Heparan sulfate (HS) represents a substantial portion of glycans that perform essential physiological functions. Heparin, a special form of HS, is a commonly used anticoagulant drug. The wide range of biological functions of HS attract considerable interest to exploit heparin or heparin-like molecules for the development of anticanccer, antiviral drugs and a better anticoagulant drug. The wide range of biological functions of HS attract considerable interest to exploit heparin or heparin-like molecules for the development of anticanccer, antiviral drugs and a better anticoagulant drug. Heparin is currently isolated from pig intestine. A contaminant in the heparin could lead to severe allergic reactions and deaths as it happened recently in US and Germany. Thus, a cost effective method for preparing synthetic heparin is highly desirable.

Methods: We have developed an enzyme-based approach to synthesize heparin and heparan sulfate. There are total of 13 specialized sulfotransferases and one epimerase involved in the biosynthesis of HS. We have expressed most of these enzymes in E. coli, permitting the access of a large amount of proteins. A low cost sulf donor system was also successfully coupled with the synthesis, which reduced the cost of the synthesis by more than 1000-fold.

Results: Our method has demonstrated the feasibility of the synthesis of the heparan sulfates with different biological functions in multi-milligram scales. This method was employed to identify novel structures of anticoagulant HS, known as Recompan. In addition, using structurally based mutagenesis approach, we are able to alter the substrate specificities of sulfotransferases. The engineered sulfotransferases allowed to synthesize those polysaccharides that can not be achieved by wild type proteins. Our results have demonstrated the potential of the enzymatic approach to prepare HS-based therapeutic agents.
Intrinsic antibiotic resistance mechanism of Mycobacteria

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Mycobacteria including Mycobacterium tuberculosis are naturally resistant to most common antibiotics and chemotherapeutic agents. The underlying molecular mechanisms are not fully understood. The mycobacterial cell wall, with its unique chemical composition and physical structure, plays a major role in the natural resistance. However, our studies of hypervirulent mutants suggest that other mechanisms also exist. I will discuss the role of these factors in the natural resistance of mycobacteria. Understanding the molecular mechanisms of natural resistance may provide insights into the development of new generation of antitymicbacterial agents or novel combinations of existing drugs.

Analgesic Activity of Dragon’s Blood Caused by Interaction of Its Components—Cochinchinenin A, Cochinchinenin B, and Loureirin B

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Background: Dragon’s blood is a renowned traditional medicine with analgesic activity. Elucidation of mechanism and material basis for its analgesic effect is important to research and development of little side-effect analgesic medicine. Aims: 1) To clarify analgesic mechanism of dragon’s blood. 2) To identify its corresponding material basis. 3) To evaluate interaction between components of dragon’s blood in producing analgesic effect.

Methods: Cochinchinenin A, cochinchinenin B and louverin B were components extracted from dragon’s blood. According to their percentage contents in dragon’s blood, two or three component combinations were prepared. Using patch clamp technique and microelectrode extracellular recordings, effects of dragon’s blood and its component combinations on voltage-gated sodium currents in dorsal root ganglion neurons and on noxious stimulation evoked discharges of wide dynamic range (WDR) neurons were evaluated. According to patch clamp technique and microelectrode extracellular recordings, effects of dragon’s blood and its component combinations on voltage-gated sodium currents in dorsal root ganglion neurons and on noxious stimulation evoked discharges of wide dynamic range (WDR) neurons were evaluated. Results: Both dragon’s blood and its component combinations not only modulated tetrodotoxin-sensitive and tetrodotoxin-resistant sodium currents but also inhibited discharge frequencies of WDR neurons. Only combined effects of cochinchinenin A (0.38mmol/L), cochinchinenin B (0.19mmol/L) and louverin B (0.08mmol/L) were similar to effects of dragon’s blood (0.05%). Inhibition rates of combined and dragon’s blood on discharge frequencies were (29.79 ± 3.72)% and (30.50 ± 3.05)% respectively. The combined effects were defined as synergistic.

Conclusions: 1) Dragon’s blood interferes not only with transmission of pain in primary sensory neurons but also with processing of pain in spinal dorsal horn. 2) Analgesic activity of dragon’s blood was caused by synergistic interaction of three components—cochinchinenin A, cochinchinenin B, and louverin B. Authors’ disclosure statement: The experimental results that the effects of dragon’s blood and its component combinations on noxious stimulation evoked discharges of wide dynamic range (WDR) neurons in spinal dorsal horn of SD rats have not been reported. Due to space limitation, only the numerical results obtained in this part have been included. In addition, our study further finds that the inhibition of dragon’s blood on capsaicin (CAP)-activated currents and CAP-evoked depolarization was greater than that of cochinchinenin B. It is inferred that the above inhibition may correlate with the analgesic effect of dragon’s blood and the combination of three components may have antagonistic effect on modulation capsaicin receptor similar to dragon’s blood.

Monitor of Chemosensitivity by Bcl-2 Transcript Kinetics in Acute Myeloid Leukemias

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Background: Enforced bcl-2 gene expression suppresses apoptosis and confers resistance to anticancer drugs. Aim 1) to develop the real time quantitative PCR that can measure the expression of the bcl-2 transcript in the therapeutic course of AML patients; 2) to analyze the association between the bcl-2 gene expression and clinical chemosensitivity in acute myeloid leukemia.

Methods: A total of 53 patients with acute myeloid leukemia were evaluated in this study. Mononuclear cell fractions were isolated from the peripheral blood of 5 patients and classified as T-cells, B-cells, granulocytes. A) Standard curve construction: The standard curve for bcl-2 transcripts was constructed and the house-keeping gene β-actin was prepared as an endogenous reference. B) Real time quantitative TaqMan PCR was performed in the ABI Prism 7700 Sequence Detector System. C) Statistical analyses were performed using MS Excel 7.0 computer software.

Results: A quantitative RT-PCR assay of the bcl-2 gene, using TaqMan™ fluorogenic detection system was developed, which detected accurately the changes of the bcl-2 in the course of the chemotherapy for AML patients. The bcl-2/β-actin ratio from the patients with AML was various, but not related to FAB subtypes. This transcript ratio was not affected by mononucleated cell types. The rapid decrease of the bcl-2/β-actin ratio in samples by the real time quantitative PCR substantiated the early response, remission induction and the susceptibility to the chemotherapy protocols (Figure A & B). In the opposite, the gradual elevation of the bcl-2/β-actin ratio demonstrated drug resistance in patients (Figure C & D).

Conclusions: By applying real time PCR to clinical samples, although the bcl-2/β-actin ratio was not related to FAB subtypes, the changing data following remission induction therapy clearly reflected drug-sensitivity. These results suggest that RT-PCR assay monitored the efficacy of the chemotherapy by quantifying the bcl-2 gene transcript in AML.

Matrix metalloproteinases at BBB and beyond in Multiple Sclerosis and HIV-dementia. New perspectives for therapeutic interventions

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Matrix metalloproteinases (MMPs) are extracellular zinc-dependent neutral proteases that play an important role in physiological processes that involve tissue remodelling. Recent evidence suggested that dysregulation and imbalance between MMPs and endogenous tissue inhibitors of MMPs (TIMPs) might contribute to different pathological conditions. Among MMPs, the subfamily of gelatinases seems to be involved in mechanisms of T-cell migration into the CNS, blood-brain barrier (BBB) disruption and demyelination in the course of MS and HIV-associated neurological diseases such as AIDS Dementia Complex (ADC).

Given the importance of MMPs as key mediators in the pathogenetic mechanisms of MS and ADC, targeting MMP enzyme activity may constitute a novel therapeutic strategy in the treatment of these diseases. We studied the role of MMPs as key mediators in the pathogenesis of MS and ADC, with particular attention to the effect of therapy on MMP secretion and expression. By using an in vitro model we investigated whether IFN-γ as well as the antiretroviral drugs zidovudine (AZT) an indinavir (IDV), drugs used for the treatment of MS and HIV-infected patients, respectively, are able to modulate the activity and the expression of MMPs in glial cell cultures. As assessed by gelatine-zymography and RT-PCR, we observed a dose-dependent inhibition of MMP-9 activity and expression in both LPS-activated astrocytes and microglia. MMP-9 inhibition by IFN-γ and antiretroviral drugs was observed only in astrocytes but not in microglia.

On the bases of these in vitro results we also investigated the effect of antiretroviral therapy on the release and the expression of MMP-9 from circulating peripheral blood mononuclear cells (PBMC) from HIV-infected individuals. By using a sensitive fluorescence-activated substrate conversion (FASC) assay we demonstrated the presence of active MMP-9 in PBMC supernatants from HIV-infected patients naive for antiretroviral therapy (ARR). By contrast, in both healthy donors and ARV-treated subjects, there was no MMP-9 net activity, indicating that MMP-9 was completely blocked by binding to its natural tissue inhibitor TIMP-1. These results outline the possibility to use antiretroviral drugs and compounds with anti-inflammatory properties, which have been shown to inhibit MMP function, for the experimental treatment of neurological disorders in which the inhibition of MMP could have clinical benefits.

All abstracts are listed in alphabetical order of the presenting author.
Inhibition of HIV-1 through an Innate Humoral Mechanism – a Potential for Vaccine Development

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Background: In prior studies we show that naturally occurring IgM anti-leucocyte autoantibodies (IgM-ALA) bind to certain leucocyte receptors e.g. CD3, CD4, CXCR4 and CXCR3 but not to others e.g. CD8, CD28 and HLA. IgM-ALA inhibits cell function, chemokine binding to receptors and chemotaxis of leucocytes. Using monoclonal IgM, derived from umbilical cord B cell clones, we show that <1% clones have IgM-ALA activity and <1% clones bind to CD4 indicating that binding of IgM to receptors is highly specific. Umbilical cord sera contained IgM-ALA but not IgG-ALA. We wanted to determine if IgM-ALA by binding to co-receptors, inhibits HIV-1 entry into cells.

Methods: IgM and IgG was purified from 10 normal and 25 HIV sera using a specific exclusion column chromatography and contaminating IgG from purified IgM was absorbed out.

Results: Physiological doses of purified individual IgM, but not IgG and not IgM pre-absorbed with leucocytes, from normal and HIV-1 sera inhibited (>95%) both X4 and R5 HIV-1 from infecting PHA+IL2 activated human PBL in-vitro and in vivo in human PBL-SCID mice. HIV-1 infectivity of PBL was also inhibited with human monoclonal IgM and IgG (2 clones) having anti-CD4 activity but not with IgM (6 clones) that lacked binding to leucocytes indicating that IgM mediated inhibition of HIV-1 is highly specific. We show that IgM-ALA inhibits HIV-1 attachment to core receptors i.e. CD4, CXCR4, CCR5 and CXCR3. Inhibition of HIV-1 synctia formation (by >90%) and inhibition of infection of R5 and X4 pseudotyped HIV-1 by IgM from certain individual HIV-1 sera were not inhibitory to some R5-HIV-1 viral strains and did not bind to CCR5 receptors indicating that certain HIV-1 may lack antibodies reactive to strain specific co-receptor epitope.

Conclusion: An innate humoral mechanism which is present from birth i.e. IgM-ALA, has a role in inhibiting HIV-1 viral entry into cells. Developing strategies to enhance in-vivo IgM-ALA e.g. through a vaccine, could prolong the asymptomatic state in HIV-1 infected individuals. This work has been published (J of Immunol, 2008; 180: 1769).

Multiple antibiotic resistance of heterotrophic bacteria from Siberian lakes as an indicator of anthropogenic influence on the ecosystems

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Background: Antibiotic usage in medicine practice and veterinary has led to an emergence of bacteria with multiple antibiotic resistance (MAR) among natural aquatic bacteria. Aim: To examine the effect of anthropogenic impact on appearance of MAR by heterotrophic bacteria isolated from two lakes differing in anthropogenic impact level and bacterial plasmid profile.

Methods: Heterotrophic bacteria isolated from central and resort/shore-line parts of Lake Shira/Lake Shunet (Southern Siberia) were investigated. The method of replica plating was used for antibiotic sensitivity testing. The following antibiotics were used: amikacin (30 µg/ml), ampicillin (50 µg/ml), benzylpenicillin (10 µg/ml), cefotaxime (25 µg/ml), doxycycline (30 µg/ml), gentamycin (10 µg/ml), kanamycin (30 µg/ml), streptomycin (30 µg/ml). Isolation of plasmids was carried out by the method of alkaline lysis.

Results: The antibiotic resistance profiles of heterotrophic bacterial isolates recovered from two lakes were determined. Resistance was detected at least in one strain for seven of the eight antibiotics tested, the exception being amikacin. No bacteria with single antibiotic resistance were found. Resistance was more frequently observed among isolates recovered from within the proximity to a tourist resort (Lake Shira), or the line (Lake Shunet) than in the samples from the centre of each lake. Bacteria with multiple antibiotic resistance (resistant to 2 and more antibiotics) were checked on a presence plasmid in their cells. We found plasmids of varying both in sizes (from less than 2.3 to >23.1 kb) and their number (from 1 to four per cell). Plasmids of medium size (about 23.1 kb) predominated in bacterial cells. The same plasmid profile was found for bacteria isolated from the central and from the resort parts of Lake Shira and these bacteria had the same antibiotic resistance pattern.

Conclusions: Multiple antibiotic resistance of heterotrophic bacteria studied is due to an anthropogenic impact on the ecosystem and this property of bacteria can be used for ecological monitoring on the lakes. One of the possible reasons of the plasmid distribution among bacteria may be plasmid capture by bacteria under human activity conditions.

Identification of the cocaine binding site on the dopamine transporter

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Background: Cocaine is a widely abused substance with psychostimulant effects attributed to inhibition of the dopamine transporter (DAT), Currently, there is no medical treatment against cocaine abuse and addiction. Interestingly, analogues of benzomorphone (BZTs) are less effective than cocaine as behavioral stimulants, despite having similar or higher affinity and selectivity for the DAT than cocaine. In addition, some BZTs have shown to antagonize the effect of cocaine in neocortical behavioural models.

Methods: We use molecular docking models dopamine, cocaine and BZTs to assess the binding site of the compounds in the DAT. The models were validated experimentally using systematic mutagenesis of the isolated residues and studying the effect on substrate affinity. We also assessed the correctness of the docking model of the cocaine analogue, CFT, by the engineering of a zinc binding site and using chemical cross-linkers.

Results: The models of dopamine, CFT and BZTs show almost complete overlap with only a few residues shown to be unique for either compound. Systematic mutagenesis of the residues proposed to be involved completely validated the models. Trapping of the radiolabeled cocaine analogue [3H]CFT in the transporter, either by cross-linking engineered cysteines or with an engineered Zn2+-binding site that was situated extrasynaptically to the predicted common binding pocket also produced results in agreement of the docking model. In particular, the orientation of Tyr156 in TM3 showed marked differences between the models. In the model for dopamine and BZTs, a hydrogen bond is formed between the OH-group of Tyr156 and Asp79 in TM1. In contrast, docking of CFT causes a disruption of this H-bond. Disruption of this interaction (Y156F) resulted in several fold decrease in affinity for dopamine and BZTs but had no effect on the affinity for CFT.

Conclusions: Our data show the molecular basis for the competitive action of cocaine at DAT. We also demonstrate in DAT a unique binding mode for cocaine, which unlike substrates and BZTs, produce a conformational rearrangement of the binding site that disrupts a stabilizing OH-bond between Tyr156 in TM3 and Asp79 in TM1.
Antisecretory Factor (AF) - an inducible antisecretory and anti-inflammatory protein

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Background. The Antisecretory Factor (AF) is a 41 kda protein which affects ion/water transport and inflammation in the small intestine. The endogenous plasma level of AF is increased by enterotoxins and by certain food constituents such as hydrothermally processed cereals. The active site of AF is a 16-peptide (AF16) at position 36-51. We here show that the peptide also exerts effects on the central nervous system.

Methods. Intracranial pressure was measured in rats with a fiber optic transducer positioned at the posterior parts of the lateral ventricle in the brains. An elevated pressure was induced by infection with Herpex simplex virus causing encephalitis (HSE); 25 µg of AF16 (n=10) or vehicle alone (n=22) was administrated intranasally twice daily. In order to measure in vitro effects of AF16 nerve membranes from Deltors cells were mounted in microchambers and 36Cl permeability studied.

Results. AF16 rescued all rats with HSE; in contrast 90 % of animals given vehicle alone died. The effect of AF16 was probably due to its capacity to reduce intracranial pressure since a single dose of the peptide reduced the pressure to normal. In vitro pmol levels of AF16 were shown to counteract the out in 36Cl-permeability in nerve cell membranes; in contrast AF peptides lacking the active sequence had no effect.

Conclusion. The results suggest that AF affects ion/water transport not only in the gut but also in the central nervous system illustrating once more the so called gut/brain axis.

Virostatics: a new class of immunomodulators with dual antiviral and cytostatic properties to inhibit viruses and protect the immune system from hyperactivation during chronic infections

LORI F2

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Background. Chronic infections are characterized by continued stimulation of the immune system, often resulting in variable degree of hyperactivation with consequent partial or complete immune exhaustion, as it is the case for HIV/AIDS.

Methods. We have characterized drugs or combination of drugs carrying both antiviral and cytostatic properties, and named them virostatic drugs. In particular, we have analyzed anti-HIV compounds, and screened them for antiviral and antiproliferative properties both in activated and quiescent lymphocytes. VS411, a fixed combination of two drugs, is the leading compound.

Results. VS411 rescued all mice with HSE; in contrast 90 % of animals given vehicle alone died. The effect of AF16 was probably due to its capacity to reduce intracranial pressure since a single dose of the peptide reduced the pressure to normal. In vitro pmol levels of AF16 were shown to counteract the out in 36Cl-permeability in nerve cell membranes; in contrast AF peptides lacking the active sequence had no effect.

Conclusion. The results suggest that AF affects ion/water transport not only in the gut but also in the central nervous system illustrating once more the so called gut/brain axis.

Nimotuzumab is a humanized monoclonal antibody directed against epidermal growth factor receptors. Three patients with anaplastic astrocytoma (AA) and 8 with glioblastoma multiforme (GBM) were intended to be treated with 3 mg of mAb labelled with 10 or 15 mCi of 188Re. In patients treated with 10 mCi (n = 6) transitory worsening of pre-existing neurological symptoms were observed. Two patients treated with 15 mCi (n = 4) developed early severe neurological symptoms and one also developed late severe toxicity (radionecrosis). In the group treated with 10 mCi, 1 GBM patient died in progression 6 months after the treatment. 2 patients (1 GBM and 1 AA) developed stable disease during 3 months. One GBM patient had partial response for more than 1 year and 2 patients (1 GBM and 1 AA) were asymptomatic and in complete response after 3 years of treatment. Maximal tolerated dose of the radioimmuno-conjugate 188Re-Nimotuzumab was 3 mg of the h-R3 labelled with 10 mCi of 188Re. The radioimmuno-conjugate showed a high retention in the surgical created resection cavity and the brain adjacent tissues with a mean value of 85.5% of the injected dose one hour post-administration. This radioimmunoconjugate may be relatively safe and a promising therapeutic approach for treating high grade gliomas.

AdCD40L Cancer Vaccine – From Experimental Models to Clinical Application

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Background. Cancer vaccines are merging as novel treatment options for cancer patients. The first line of vaccines encountered obstacles such as tumor immune escape. Novel strategies have been explored that aim to both activate anti-tumor immunity as well as hamper the regulatory mechanisms exhibited by the tumor. The objective for our tumor vaccine program was to evaluate the efficacy of AdCD40L therapeutic vaccination for solid tumors such as bladder cancer.

Methods. Adenoviral vectors were used to transfer the immunostimulatory gene CD40L into tumors. Preclinical evaluation of tumor eradication and development of anti-tumor immunity was made in our murine bladder cancer model as well as in both murine and human culture systems. Clinical evaluation was made in patients with bladder cancer in a clinical phase II/III trial. Tumor immunity upon therapeutic vaccination was investigated with techniques such as CBA, quantitative PCR, proliferation assays, and flow cytometry.

Results. In a series of publications we have demonstrated that AdCD40L can cure highly aggressive bladder cancer in experimental models. In these models, AdCD40L was shown to efficiently activate tumor-specific immunity by maturing dendritic cells, stimulating Th1 cytokines and activating cytotoxic T cells. Furthermore, the levels of T regulatory cells and suppressive cytokines such as IL-10 and TGF-b were decreased which may be crucial for the anti-tumor effect. In recently obtained unpublished animal data, local AdCD40L administration into tumor-positive bladders eradicated both bladder tumors and distant lung metastases. Currently, a clinical evaluation is performed. Phase I is completed (n=15) and AdCD40L vaccination was safe. No side effects have been documented. Routinely, bladders are removed from patients with high-grade malignancy to avoid incurable metastases. After AdCD40L therapy no high-grade tumor cells could be detected in the cystercentomized bladders. Further, T regulatory cells were reduced after treatment in compliance to our experimental models.

Conclusion. AdCD40L cancer vaccination seems to eradicate high-grade malignant tumor cells and is a promising candidate for therapeutic vaccination of both local and disseminated malignancy.
Effects of Theranekron (alcoholic extract of Tarantula cubensis) in treatment of Foot- and-mouth disease (FMD) lesions in cattle

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Background: Foot- and Mouth Disease (FMD) is an acute viral disease of all cloven-footed animals. In enzootic countries where a slaughter policy is not in force, treatment of infected animals is recommended. Theranekron (Richterpharma, Austria) contains the whole extract from spider tarantula cubensis. In the present study therapeutic effect of Theranekron on foot- and-mouth disease in cattle was investigated.

Methods: During an outbreak of FMD in cattle in Iran, fifty infected cattle (in early stage of disease) treated with single subcutaneous injection of Theranekron (treatment group). Fifteen infected animals at the same time chose and treated as control group (control). The results of treatment in two groups on oral mucosal lesions of infected cattle has been appeared. A significant difference between median value of oral lesions in treatment and control groups were shown in 24 hours and days 3, 4 after treatment by Mann whitney test (P<0.05).

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Conclusions: From results of present study it appears that Theranekron is a very efficient drug for treatment of FMD cases in cattle especially because of its anti inflammatory and healing properties and convenient in use (single injection) compare to the routine treatment.

Proposed antiangiogenic agents with mechanisms that prevent tumor cell survival and resistance to cytotoxic therapies

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Background: The transcription factor hypoxia inducible factor-1α (HIF-1α) facilitates cell survival and angiogenesis through transcriptional activation of multiple target genes including vascular endothelial growth factor (VEGFR). HIF-1α has also been linked to increased drug resistance through expression of the ABCB1 and ABCC1 transporters preventing intracellular drug accumulation. We recently found that loss of methylation-controlled J protein (MCJ) correlates with increased expression of ABCB1 and increased resistance. Others have also shown that increased expression of the J protein kinases (JNK) is regulated by MCJ through sites phosphorylated by GSK3β.

Results: Inhibition of MCJ expression or GSK3β activity, showed drug resistance and overexpression of GSK3β by means of PI3 kinase inhibitors as therapy to prevent angiogenesis and drug resistance in cancer cells.

Conclusions: This study illustrates a mechanism in which posttranslational modification by GSK3β attenuates HIF-1α possibly through MCJ interaction, ubiquitination and proteasomal degradation, thus decreasing angiogenic potential and preventing drug resistance. We propose the future study of GSK3β activation by means of PI3 kinase inhibitors as therapy to prevent angiogenesis and drug resistance in cancer cells.

**Cardioprotective Anthracycline PKC Activators For The Treatment of Drug-Resistant Tumors**

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Successful cancer chemotherapy is often limited by cellular drug resistance, which requires dose escalation for circumvention, and by systemic adverse effects, which limit the cumulative dose of drug administered. Typical of these dual limitations are the anthracycline antibiotics, such doxorubicin (DOX), whose cytotoxicity is blocked by multiple resistance mechanisms, including the expression of multidrug transporters (MRPs-1, P-gp), anti-apoptotic proteins (Bcl-2, Bcl-XL), proliferative proteins (NF-κB, Bcr-Abl kinase), and by p53 protein dysfunction. DOX efficacy is also limited by well-characterized and often irreversible cumulative toxicities linked to the generation of reactive oxygen species (ROS) by the anthraquinone ring.

In response to these therapeutic impediments, we have developed a novel class of anthracyclines, represented by Al-benzyladramycin-14-valerate (AD 198) and Al-benzyladramycin-14-pivale (AD 445). AD 198 and AD 445 are markedly more lipophilic than DOX and, consequently, circumvent efflux by multidrug transport proteins. Unlike DOX, AD 198 and AD 445 localize in the cytoplasm and do not target DNA but, rather, bind to the CTb (diacylglycerol-binding) domain of protein kinase C (PKC).

Drug-mediated PKC-delta activation triggers rapid apoptosis in proliferating cells through a novel mitochondrial-dependent pathway and in a manner that circumvents the anti-apoptotic effects of Bcl-2 and Bcl-XL expression. Since apoptosis is triggered rapidly and without the requirement of cell cycle arrest, enhanced proliferative signaling or p53 dysfunction do not block AD 198/AD 445 mediated-apoptosis.

Despite the retention of an anthraquinone ring, AD 198 is non-cytotoxic in the breast cancer (chronologically-dosed) model MCF-7. This compound was the activation of PKC-epsilon by AD 198 and enhanced cardioprotective signaling in cardiomyocytes, which protects the heart from reperfusion injury following induced ischemia or from high-dose DOX-induced injury in an in vivo perfused heart model. Our studies suggest that AD 198 and AD 445 may provide improved therapy for drug-resistant tumors without concern for dose-limiting toxicities associated with conventional anthracyclines or with cardioprotection when administered in combination with potent cardiototoxic antitumor agents.

A Potential Anti-Cancer Drug From a Plant Extract, Tillandsia recurvata

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1Environmental Health Foundation; University of Maryland (HVF) School of Medicine

Background: Most successful cancer drugs have been derived either directly or indirectly from plant materials. Jamaica is fortunate to have 84 of the 120 recognized medicinal plants of the world. One of these plants, Tillandsia recurvata has demonstrated potent anti-cancer activities both in vitro and in vivo. The following specific activities were documented: 1) Isolation and purification of bioactive compounds from this plant extract; 2) These compounds were tested in vitro for anti-cancer properties and in vivo activities of this isolated compound; and 3) Assessed through preliminary findings one of the mechanism of action of this compound in vivo.

Methods: Tillandsia recurvata is a flowering plant native to tropical areas of the New World. The fresh plant material was dried in steam oven at 70°C then macerated and then extracted with cold water. Various fractions were then isolated be column chromatograpy. Subfractions were then processed and tested via bioassayed fractionation. This was followed by using advanced separation technology (HPLC, supercritical fluid chromatography and capillary electrophoresis). This was followed by LC/MS and finally NMR.

This study involves the testing of the isolated fractions against 5 different histogenic tumors invivo using the transplanted nude mouse models. The five mouse cell lines used in these assays were the following: 1) melanoma; 2) prostate; 3) breast; 4) gliosarcoma and 5) b-cell lymphoma. The extract was tested invivo against the above tumor cell lines. The in vitro studies included 10 mice per group and using the crude form of the extract at 10mg per mouse per day for 7 days. This was done for each of the above tumor cell lines compared with controls with normal saline treatment. The purified compound is now currently being produced at ground levels to test invivo.

Results: Utilizing the bioassays-guided fractionation process, the bioactive moiety was isolated at 98% purity. This purified compound was tested invivo and demonstrated to be highly effective at a rate of 95% to 100% cell kill in the invivo assays of 5 different histogenic tumor cell lines. The invitro studies were equally as impressive utilizing the crude extract. All tumors responded to the treatment by reducing the tumor size from 4mm X 4mm to almost non existent on gross examinations of all the above tumor cell lines. On histology 90% to 95% of the tumors were undergoing cell death. Using immunohistochemical staining we were able to determine that the cell death was due primarily to induced apoptosis. No toxic signs or systems were observed in any of the invitro studies.

Conclusions: This newly extracted compound demonstrated a significant anti-cancer properties. Preliminary studies from this newly isolated compound indicate that this compound may serve as an excellent new anti-cancer drug.

**Authors’ disclosure statement** This compound has been patented.
A New Molecular Mechanism of Action of a Leading Chemotherapeutic Drug—Cisplatin and Its Novel Applications

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**Background:** There is pressing need for mechanistic understanding of action of existing anticancer drugs at the molecular level, which can, in turn, lead to molecular-mechanism-based design of new anticancer drugs. Despite its great success in treating certain cancers, cisplatin as the most effective chemotherapeutic drug has severe toxic side effects and both intrinsic and acquired resistance. Such drawbacks have even prompted the call to discontinue the therapeutic applications of cisplatin-like anticancer drugs. One outstanding problem is that the precise mechanisms of action of these anticancer drugs remain elusive. Aims: 1) To obtain a true understanding of the molecular mechanism of action of cisplatin. 2) To optimize cisplatin chemotherapy. 3) To apply our mechanistic understanding of cisplatin to developing new effective cancer therapies, including combinations with radiotherapy and photodynamic therapy.

**Methods:** Time-resolved ultrafast (femtosecond) laser spectroscopy is the most powerful technique for real-time observations of molecular reactions since it uses laser flashes of such short duration down to the time scale on which the reactions actually happen – femtoseconds (fs) (1fs = 10^-15 second). The molecular mechanism of anticancer drugs revealed by this technique is examined with biomedical methods such as DNA damage and cell death measurements.

**Results:** An extremely high reactivity of cisplatin with electrons and a new electron transfer mechanism of the cisplatin-DNA interaction have been discovered. Furthermore, we have revealed the molecular reaction mechanism of the combination therapies of low-dose cisplatin with radiotherapy and photodynamic therapy. Based on our mechanistic understanding of cisplatin, new molecular regulators have been developed to enhance the therapeutic effects and to reduce the side effects.

**Conclusions:** 1) The therapeutic effectiveness of cisplatin is closely related to its high reactivity with electrons. 2) This finding can be utilized to improve the chemotherapy with cisplatin and to develop new combination therapies for effective treatment of cancer.


Authors’ disclosure statement: Financial support from the Canadian Institutes of Health Research (CIHR) is acknowledged.
Background: Cyclic nucleotide phosphodiesterases (PDEs) play a key role, downstream receptor activation, in intracellular signalling by selectively hydrolysing cyclic nucleotides that serve as second messengers in a number of cellular pathways. Eleven PDE isozyme families (> 100 proteins) have been characterized and are differentiated by their substrate specificity, their tissue, cell and subcellular distributions, and also by their short-term and long-term regulations. Altogether, the complexity of these families allows a fine and compartmentalized regulation of cyclic nucleotide levels. Among these families, PDE2, which hydrolyzes both cAMP and cGMP and whose the cAMP hydrolysis is stimulated by cGMP, and PDE4, which specifically hydrolyses cAMP, represent the main isozymes in human umbilical vein endothelial cells (HUVECs). Angiogenesis is defined as the formation of new blood vessels from pre-existing ones. Since angiogenesis plays a major role in tumor development induced by tumoral vascular endothelial growth factor (VEGF) secretion, nowadays antiangiogenic efficient therapeutic approaches are developed mainly at the receptor level.

Methods: Herein, by using an in vivo angiogenesis model (HUVECs), an in vivo angiogenesis model (chicken embryo chorioallantoic membrane; CAM), and an in vivo tumourisation model (tumor growth induced by BF16/10 cells in 20 C57BL/6 mice), we show that the combination of PDE2 (EHA4) and PDE4 (RP73401) inhibitors overcome angiogenesis.

Results: Our studies show that VEGF-induced HUVECs proliferation and migration is inhibited with PDE2 and PDE4 upregulations (mRNA, proteins and activities) and that PDE2 and PDE4 inhibition increases cAMP level, inhibits cell migration and proliferation and also inhibits VEGF-induced cell cycle progression at the level of E2F phosphorylation, cyclin D1 expression. Similar studies performed with delphinidin (a grape polyphenol which inhibits PDE2 and PDE4), show that delphinidin inhibits in vitro (HUVECs) and in vivo (CAM) angiogenesis. Tumor growth treated with EHA4+RP73401 is reduced by 30% (P=0.014).

Conclusions: It clearly appears that targeting VEGF-upregulated endothelial PDE2 and PDE4 is a new and original strategy to overcome the intracellular signalling dysfunction induced by VEGF stimulation which should induce less side effects.

Anti-HIV activity of lectins from marine invertebrates

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Background: Role of HIV envelope carbohydrates and target T-cells glycocalix is very important for understanding of mechanism of virus invasion processes. Aims: To check anti-HIV activity of lectins from marine invertebrates. Methods: The data were obtained in FACSA analyser using Jurkat T lymphocytes, specific for CD18 antigen, treated with anti-CD18 scFv antibody for 50 hours. Conclusions: (1) The scFv agent is shown to bind CD18 antigen and the epitope mapped to the beta 2 domain. 2) Anti-D18 scFv significantly blocks leukocyte infiltration into major organs (liver, lung) and attenuates the release of proinflammatory cytokines (TNF-a, IL-6) in systemic sepsis conditions. 3) Animals treated with the scFv could survive longer compared with those from the placebo group in the CLP model. Collectively, our findings suggest that blocking the CD18 beta 2 domain by anti-D18 scFv promises to be an effective approach for therapeutic intervention of leukocyte-mediated tissue damage.

Authors' disclosure statement: This study is supported by the Research Grants Council of Hong Kong and has been filed for US Patent (No.: 61/080,568).

Anti-HIV activity of lectins from marine invertebrates

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
the most profound regression of elevation by more than 7.5% together with effective LDL-C lowering resulted in and natural compounds activate – via for new agents with potential to regress atherosclerosis (1-3). Many xenobiotics mediated through the actions of P450s, PXR – mechanisms which eliminate excess cholesterol. The antiatherogenic mechanisms regress atherosclerosis and the manifestation of atherosclerotic disease, particularly coronary heart disease (CHD), the leading cause of death in the world.

Results: Several compounds upregulate genes acting in cholesterol elimination such as apo AI, ABC (ATP-binding cassette) transporters and P450s. P450s including CYP7A1, CYP27A1 and CYP46A1 generate hydroxycholesterols which promote the activation of cholesterol-eliminating mechanisms (see 1-3). The progress in studies on cholesterol regulation has greatly stimulated the search for new agents with potential to regress atherosclerosis (1-3). Many xenobiotics and natural compounds activate – via nuclear receptors including LXR, PPAR and PXR – mechanisms which eliminate excess cholesterol. The antiatherogenic effects of many compounds including statins, fibrates and cholestyramine are mediated through the actions of P450s. Rosuvastatin therapy which effectively reduced LDL-C and apo B and raised HDL-C and apo AI resulted in a significant regression of atherosclerosis. The increases in HDL-C levels were found to be an independent predictor of a beneficial outcome with statin therapy (2,3).

Conclusions: P450 enzymes are essential in the maintenance of cholesterol homeostasis. Effective gene-activating agents upregulating cholesterol-eliminating mechanisms regress atherosclerosis and reduce the occurrence of fatal and non-fatal CHD and other cardiovascular events.

Cytotoxic Platinum(II) Complexes With Quateryridine Ligands As A New Class Of Topoisomerase I Inhibitors

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Background: Topoisomerase I (Topo I) has been receiving considerable interest due to its capability in regulation of cell division. The spatial arrangement of DNA before, during, and after replication is essential for cell division process. Topoisomerases regulate the linking number, twist, and writhe, ensuring that it is arranged adequately for DNA replication by breaking one or two strands of the DNA. Thus inhibition of topoisomerase by ternary DNA-intercalator-Topo complex formation is important in the context of drug discovery for anti-cancer treatment.

Methods: The interactions of 

\[ [Pt(dqP)]_2(CF_3SO_3)] \]

[1, dqP = 4,4'-diphenyl-2,2'-6,2''-terquatyrildine] with double-stranded DNA was examined by spectroscopic, electrochemical, and hydrodynamic methods. The spectroscopic data were analyzed with McTheo, van Hhoff, and Gibbs-Helmholtz equations. The binding mode of Pt towards the Topo-I-linked DNA was further studied by molecular modeling.

Results: The binding of 1 to calf thymus DNA led to increases in the DNA melting temperature (Tm, ~7°C), modest hypochromism (12% of the absorption band at λmax of 260 nm). The binding constant of 1 with DNA, as determined from the melting temperature, is 1.6 ± 0.2 M−1. A modeling study on the interaction between 1 and Topo-linked DNA revealed that 1 intercalates to Topo-DNA in a similar manner to topotecan (known topoisomerase I inhibitor), and exhibits a strong binding interaction. As determined by MTT assays, 1 exhibited moderate cytotoxicities toward several human cancer cell lines (KB-3-1, HeLa, Hela). According to confocal microscopic and flow cytometric studies, 1 induced apoptosis (70%) in cancer cells with ~5% necrosis detected. Notably, 1 at concentrations > 25 M inhibited the Topo I-mediated relaxation of DNA.

Conclusions: Cyclo metallated platinum(ii) complex with quateryridine ligand binds to DNA with binding constants ~10^5 M−1 and exhibit comparable cytotoxicities toward a series of human carcinoma cell lines with cisplatin; can induce apoptotic cell death in human carcinoma cells presumably by stabilizing the ternary DNA-intercalator-Topo complex.

Calcium-Releasing Agent Exhibits Bioactive Effects In Endodontic Therapy

MAEDA H 1, TOMIKO Y 1, FUJI S 1, WADA N 2, MONNOUCHI S 3, HORI K 3, AKAMINE A 1

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Background: Mineral trioxide aggregate (MTA) is a recently developed endodontic biomaterial. The materials with a faster setting time and the smaller amount of water required are often preferred. It is of interest to study the differences of MTA with respect to setting time and water amount.

Methods: Two HPLF populations were isolated and generated from the harvested bone samples of 25-year-old female and a 14-year-old male who visited Kyushu University Hospital for extraction, and the cells were maintained in 10%FBS/MEM (Gibco-BRL, Grand Island, NY). All procedures were performed in compliance with the regulations of Kyushu University. White PreRoot MTA (DENTSPLY Tulsa Dental, Johnson City, TN) was mixed with sterile water according to the manufacturer’s instructions, dispensed into plastic lids of 1.5ml micro-centrifuge tubes, and placed in a humidiﬁed incubator (37°C) for 12hr. MTA discs (4mm and 1mm thickness) were then rinsed with -MMEM, placed in 24-well culture plates (10disc/well), and subjected to the cultures with HPLF.

Results: SEM observation showed attachment of HPLF onto MTA discs within 24hr. MTA did not inhibit the cell proliferation, and furthermore up-regulated expression of bone-related genes, osteopontin and osteocalcin in HPLF within 14days, and eventually induced mineralization in HPLF around MTA after 4wk of culture. MTA furthermore exhibited calcium release into the culture media. Then CaCl2 treatment not only stimulated mRNA expression of osteopontin and osteocalcin, but induced mineralization in HPLF, while Mac2 treatment did not show any significant effects. Consequently, HPLF treated with MTA and CaCl2 definitely increased bone morphogenetic protein 2 (BMP2) gene expression.

Conclusions: 1) MTA possesses the biocompatibility for HPLF. 2) MTA induces the osteogenic differentiation of HPLF through up-regulated expression via the calcium release. Therefore, 3) MTA is a bioactive material in endodontic treatment.
Background: Drugs targeting the renin-angiotensin system (RAS) are an important therapeutic class for the management of several cardiovascular disorders. Aliskiren is the first approved orally active renin inhibitor and exhibits a dose-dependent antihypertensive effect. The purpose of this study is to develop a semi-mechanistic pharmacokinetic-pharmacodynamic (PK-PD) model to evaluate aliskiren effects on several biomarkers of RAS in humans.

Methods: Mean plasma renin activity (PRA) and plasma concentrations of aliskiren, active renin (AR), angiotensin-I (ANG-I), and angiotensin-II (ANG-II) were extracted from a published 3-way crossover, placebo-controlled study. Healthy male volunteers (n=18) received either placebo or 20mg enalapril followed by 2 oral doses of aliskiren (40 and 80mg, or 160 and 640mg) once daily (8-day washout period). The final PK-PD model was fitted to the data in 3-stages: 1) multiple-dosing PK were modeled and fixed during subsequent stages. 2) single-dose PK were characterized, and 3) single- and multiple-dose PK were fitted jointly. All model parameters were estimated using the maximum likelihood method in SIM-ADAPT (v.1.51).

Results: A two-compartment model with nonlinear elimination and distribution best described aliskiren disposition. AR increased in a dose-dependent manner following the administration of aliskiren, which was described by an indirect stimulatory response model in conjunction with an empirical sub-model of function, in contrast to AR. PKA was described as a dose-dependent manner and was maximally inhibited within 1 hour after aliskiren administration. This response was well captured with a direct inhibitory Emax model, and the estimated aliskiren concentration producing 50% inhibition of PRA was 0.66ng/mL, which is similar to in vitro estimates (0.33ng/mL) after correcting for plasma dilution, in vivo ANG-I and ANG-II paralleled the changes in PRA, and ANG-I and ANG-II remained linearly correlated throughout the study. A reduced model was also developed excluding ANG profiles, which successfully described AR and PRA profiles after multiple-dosing.

Conclusions: An integrated PK-PD model of aliskiren was developed which is consistent with the pharmacology of renin inhibition. The final and reduced models test current hypotheses of RAS inhibition by direct renin antagonism and may prove useful in the future clinical development of renin inhibitors.

Tetracyclines: A Historical Pitfall And Additional Concept On The Treatment Of Rickettsial Diseases

MAHARA FI

1Mahara Hospital, Tokushima, Japan; 2Univ. of Fukui, Fukui, Japan

Background: A febrile disease thought to be Scrub typhus(Tsutsugamushi disease) appeared in China in 313 A.D. The first scientific report was made by Bealts and Kawabami in 1978. This old and historical disease was highlighted at the turn of the 21st century. In Japan, it is a problem to be recognized as a neglected pathogen in Orientia tsutsugamushi in. In it, the author defined the following problems. 1) This disease is still recognized as a non-infectious disease, and the estimated aliskiren concentration producing 50% inhibition of PRA was 0.66ng/mL, which is similar to in vitro estimates (0.33ng/mL) after correcting for plasma dilution, in vivo ANG-I and ANG-II paralleled the changes in PRA, and ANG-I and ANG-II remained linearly correlated throughout the study. A reduced model was also developed excluding ANG profiles, which successfully described AR and PRA profiles after multiple-dosing.

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Infectious Pregnancy Complications

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Epidemiological reports estimate that 7.7 million perinatal deaths occur annually worldwide, including 4.3 million that take place in late pregnancy, while the remaining neonates die in the first weeks of life. Reports attribute the majority of these consequences to infections of the fetus in utero. Infections during pregnancy affect the mother and often some infections may be transmitted to the fetus in utero, during the intrapartum period or, postnatally, with potentially serious consequences. Many infections are linked with increased risks of prematurity delivery and low birth weight, and associated morbidity and mortality of both mother and child. Acute or chronic specific infectious diseases may be contracted during the course of pregnancy, and conception may occur in women already subject to an infection. The coexistence of pregnancy may aggravate the risk to maternal life in cases of the more serious of these diseases. In pregnancy most infections are no more common, nor more serious than in a non-pregnant population of women of similar age. The effects on pregnancy depend on the time of conception, duration, and the stage of fetal development when it occurs. Mild exposures during the preimplantation period, and more severe exposures during embryonic and fetal development often result in miscarriage, premature labor, growth restriction, or stillbirth. Hypermethria may also cause a wide range of fetal structural and functional defects, with the central nervous system (CNS) being most at risk. While there is a greater incidence of neonatal morbidity and mortality with transmitted infections, not all maternal infections lead to transmission to the fetus, nor does transmission to the fetus lead to disease or sequelae. During the puerperium, parturient women are particularly susceptible to serious infections of the genital tract and childbed fever remains one of the most important causes of maternal death. Infections in pregnancy may be viral, bacterial or protozooal, affecting both mother and fetus. Some of the infections cause fevers, while others may not; this chapter will concentrate on infections resulting in maternal pyrexia, and some other infections which may not result in maternal pyrexia, but have important implications for the pregnancy and the fetus.
gene expression, including HO-1. Delivering heme or synthetic heme analogues to induce or inhibit heme-regulated approach to design compounds, based on the ligand-binding property, for the peptide: KYCCSRK could specifically bind hematin, which offers a rational utility as effectors of cell cycle progression and cell differentiation. Furthermore, activate, respectively, ERK, by IGF and PKC-

The Critical Functions Of Biliverdin Reductase (BVR) In Insulin/Insulin Growth Factor-1 (IGF-1) And MAPK Signaling Pathways: A Potential Therapeutic Application In Treatment Of Diabetes And Cancer

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Background: Among eukaryotic proteins, the human(h) BVR has a more diverse and expansive spectrum of functions in the cell than any other protein. The breadth of its functions was discovered in recent years, leading to the development of peptide-based technology to combat diseases that are associated with wrong folds or misfolded proteins, such as diabetes and cancer.

Methods: In vitro and in vivo experiments, using human embryonic kidney cells (HEK293) or murine fibroblasts, were performed to investigate the role of BVR in insulin receptor kinase/IGF-1/MAPK-regulated signaling and regulation of gene expression.

Results: Data gathered identified the ability of BVR, its variants and small fragments, in modulating cell signaling and, hence, the wide range of functions that are regulated by protein kinases. These functions include growth, differentiation, gene transcription and metabolism. Regulation of glucose uptake, induction of heme oxygenase-1 (HO-1), and cytokine and Toll-like receptor signaling were identified as potential target candidates for BVR-based therapeutic strategies.

Conclusions: Developing functional foods to improve public health requires contributions from on-going research and modifications to the current regulatory framework to facilitate the review of new functional components and their health claims. It is also imperative to communicate the correct information to the consumers.

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Intrapercardial Cispalatin Treatment Prevents Effectively The Recurrence Of Neoplastic Pericardial Effusion

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Background: The differential diagnosis of neoplastic (NE) vs. radiation induced pericardial effusion (RE) in malignancies is the prerequisite of an adequate intrapericardial and/or systemic treatment.

Methods: Out of 269 patients, 153 pts (64.2%) with pericardial effusion (69% m, mean age 58.8±12.3 y) were identified as NE, 15 pts (66% fem., mean age 54.7±12.4 y) as RE. The etiological assessment was highly effective because it was assisted by pericardioscopy, epi- and pericardial biopsy and pericardial cytology. Pericardial effusion and biopsy analyses included biochemistry, cytology, immunohistology, and PCR.

Results: In NE we identified: lung cancer, 52.4%; breast cancer, 19.0%; Hodgkin’s disease, 4.8%; oesophageal cancer, 3.4%; mesothelioma, 2.4%; colon cancer, 4.8%; and undifferentiated cancer of unknown origin, 14.2%. In RE 11 pts had previous breast cancer, 4 pts bronchus carcinoma but the PE was negative for them.

NE were treated with intrapericardial cispalatin (single instillation of 30 mg / m² for 24 h) in addition to the tumour-specific systemic chemotherapy. It prevented recurrence of pericardial effusion during the first 3 months of the follow-up in 92.8%, and after 6 months in 83.3% of the pts. Lung cancer patients had fewer effusion relapses at the 6 months follow-up (4.5%) than breast cancer patients (37.5%) (P=0.05). Myocardiac ischemia occurred after 1/42 cispalatin instillations, but there were no other complications.

NE received 500mg / m² transtazonolactano (Votelon A) intrapericardially followed by 6 months oral treatment with colchicin (3x0,5 mg). Recurrence of effusion was prevented in 13 of 15 cases (86.6%) after 3 and 6 months. With the Touhy needle and the Marburg Attacher we reach now small effusions for intrapericardial diagnosis and treatment.

Conclusions: 1) Intrapercardial treatment with cispalatin prevents recurrences of NE effectively. The treatment was more successful in lung than in breast cancer pts. 2) In RE scirrhous treatment with tiamicin, calcitriol was equally effective. 3) Pericardioscopy adds considerably to adequate diagnosis and consecutive treatment.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
The Thyroid Gland Function Assessment In Women After Mastectomy And Chemotherapy During Breast Cancer Therapy.

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**Background:** For many years much attention has been focused on an interaction between the breast disease and the thyroid gland sufficiently in the literature. In those studies the question whether disease changes in the thyroid gland can influence the thyroid gland function or the thyroid gland reacts to the breast disease. However, there are also works concerning the inverted question whether the breast cancer therapy, in particular after mastectomy and chemotherapy, can disturb the thyroid gland function. So, the aim of the study is to investigate the influence of the mastectomy and chemotherapy on the thyroid gland function in women after breast cancer therapy.

**Materials and methods:** 173 patients aged 30-80 (average 56) were included in this study. The studied group comprised 97 women after breast cancer therapy (average age 60). The control group consisted of 76 patients (average age 55). The thyroid gland function was assessed by measuring the hormone concentration and the level of antithyroid antibodies. (anty-TPO and anty-Tg), which lead to destruction of the thyroid gland tissue, the thyroid gland function of the women after mastectomy and chemotherapy should be monitored morphologically as well as functionally.

**Results:** The effects of PEG spacer length and ligand density on folate receptor-targeted liposomes were evaluated. In human oral carcinoma KB cells, which overexpress FR, modification with sufficiently long PEG spacer and a high concentration of folate ligand to non-PEGylated liposomes increased the FR-mediated association and cytotoxicity more than with PEGylated and masked folate-linked liposomes. On the contrary, in mice bearing murine lung carcinoma M109, modification with the folate ligand in PEGylated and masked folate-linked liposomes showed significantly higher antitumor effect than with non-PEGylated liposomes irrespective of the length of time in the circulation after i.v. injection.

**Conclusion:** We have demonstrated that due to mutual induction each bacteriocin-mediated bacteriocin induction enable producers to successfully compete and defend their niche against challenging invaders. We thus suggest that bacteriocin-mediated bacteriocin induction enable producers to successfully compete and defend their niche against challenging invaders. We thus suggest that bacteriocin-mediated bacteriocin induction enable producers to successfully compete and defend their niche against challenging invaders. We thus suggest that bacteriocin-mediated bacteriocin induction enable producers to successfully compete and defend their niche against challenging invaders. We thus suggest that bacteriocin-mediated bacteriocin induction enable producers to successfully compete and defend their niche against challenging invaders. We thus suggest that bacteriocin-mediated bacteriocin induction enable producers to successfully compete and defend their niche against challenging invaders. We thus suggest that bacteriocin-mediated bacteriocin induction enable producers to successfully compete and defend their niche against challenging invaders. We thus suggest that bacteriocin-mediated bacteriocin induction enable producers to successfully compete and defend their niche against challenging invaders. 

**Conclusion:** Taking into consideration the high level of the concentration of antithyroid antibodies: (antity-TPO and anty-Tg), which lead to destruction of the thyroid gland tissue, the thyroid gland function of the women after mastectomy and chemotherapy should be monitored morphologically as well as functionally.
Background: G-CSF is detected in follicles and it shows a peak concentration in serum on a few days before ovulation. The expression level of G-CSF mRNA in the follicular wall at similar phase is 10-fold greater than other phases. These findings indicate that G-CSF plays an important role in the mechanism of follicle rupture, ovulation. LUF is an ovulation disorder that shows follicular growth and luteinization but lacks follicle rupture. We have conducted a clinical trial to induce ovulation by G-CSF for the patients of LUF.

Methods: Patients who received Clomiphene (CC) - human Chorionic Gonadotropin (hCG) treatment for ovulation induction and shown LUF at the last induction cycle participated in this study with informed consent. In addition to CC - hCG, G-CSF 100 µg was administered at 24 - 48 hours before hCG administration in 62 cycles of 56 patients, considering the natural cyclic changes of serum G-CSF. Ovulation was then confirmed by ultrasonography.

Results: The total numbers of LUF cycles before G-CSF treatment were 59 of 130 cycles (45.4%). Ovulation was successfully induced in 54 cycles (87.1%) with G-CSF, which is significantly higher than the cycles without G-CSF (P<0.0001). Pregnancy was confirmed in 4 cases of G-CSF treated cycles.

Conclusions: G-CSF administration during CC - hCG treatment is very effective to prevent LUF. Since there are no other treatments at present, G-CSF must be used for LUF patients as the first choice.
Isoniazid: It Was Or It Is A Magic Bullet?

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In 1952, Fox (US 2596069) reported the realization of the hydrazide of isonicotinic acid (isonicotinyl hydrazide, INH). The drug was an intermediate in the synthesis of the thiosubcarbazone of isonicotinoylaldehyde, a compound created by Fox himself during his search for novel thiosemicarbazone derivatives with antimycobacterial properties. These compounds had been studied by Domagk in the 1940s, and one, 4-acetylaminothiosemicarbazone thiosubcarbazone (TB-1/698, aminothiozone, thiacetazone), had already been marketed as an antitubercular drug from 1950. The drug was an intermediate in the synthesis of the thiosubcarbazone of isonicotinoylaldehyde, a compound created by Fox himself during his search for novel thiosemicarbazone derivatives with antimycobacterial properties. These compounds had been studied by Domagk in the 1940s, and one, 4-acetylaminothiosemicarbazone thiosubcarbazone (TB-1/698, aminothiozone, thiacetazone), had already been marketed as an antitubercular drug under the name of Conteben. Previous studies on the antitubercular properties of pyridine bodies – in particular, nicotinamide – also contributed to the development of INH. Chorine (1945) had found the latter compound to possess in vivo activity against infections caused by mycobacteria in animals, and, at very high doses, a antitubercular activity was found in humans as well (1951-52). Substitutions of its heterocyclic group rendered nicotinamide inactive as an antimycobacterial drug [e.g., N-2-thiazolyl nicotinamide (1945-52), unlike those of the amido group but all of substituted forms endowed with antitubercular activity as trimino isonicotinic acid and its methyl ester were lacking in vitamin properties. Relatively little attention had been focused on the hydrazine fraction of INH. The benzalbenzene hydrazide had displayed in vitro activity at a concentration of 10^-6 M. The hydrazides of nicotinic acid and its derivatives had very limited (but structurally interesting) in vitro but were inactive in vivo. The fundamental mechanism of action of isoniazid is the inhibition of pyruvate dehydrogenase. INH proved to be an almost ideal antitubercular drug. In addition to being fully selective, the drug has never been exceeded by other anti-TB drugs. It displayed maximum activity in vivo at a few days of tuberculous after a few days of treatment, and could be given during pregnancy. Both the frequency and severety of its adverse effects (hepatic, peripheral neuropathy that could be managed with vitamin B6 supplementation, CNS effects, immune, allergic, and hematologic disturbances) were more than acceptable. Given its activity at the CNS level, INH therapy was often proposed for certain neurologic disorders. INH was (and is) a drug fully deserving of the appellation ‘magic bullet’.

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Background: Substances and methods used to increase the oxygen blood transport and the athlete’s performance can be detected but the screening phase performed by International Federations remains a critical issue. The project A.R.I.E.T.T.A. aimed to develop a software able to analyze athletes’ haematological and performance profile and to point out those reflecting an abnormal pattern.

Methods: 120 Athletes belonging to the International Biathlon Union gave their written informed consent to the study. The haematological and performance data, previously collected were used to develop the A.R.I.E.T.T.A. software.

Results: The software includes the following sections: 1) Log-in 2) Data-Entry: data can be loaded, stored and grouped 3) Analysis: data can be analysed, validated scores calculated, parameters displayed simultaneously as statistics, tables, graphs, individual or subpopulation profiles 4) Scanning: an immediate evaluation of the risk score of the present sample and/or the athlete under study can be obtained. The risk score is calculated combining different parameters, absolute values and inter-intra-individual variations considered concurrently with different weights.

Conclusion: A.R.I.E.T.T.A. software enables a quick evaluation of blood results, favouring surveillance programs and timely target testing controls on athletes by the International Federations. Future studies aiming to validate the risk score and to improve the diagnostic phase will enable an upgrade of the system.

Enhanced Depot Vaccine Formulations, Vaccinoma® And Depovax™. For Cancer And Pandemic Influenza Applications

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Background: The development of a vaccine that can induce a robust cellular and humoral antigen specific immune response after a single dose would be ideal for pandemic flu and cancer immunotherapy. IVT has developed two novel depot vaccine formulations, Vaccinoma® and Depovax™ which induce enhanced immune responses when compared to current common treatments.

Methods: To detect humoral responses induced by the recombinant H5 antigen (Vietnam/04), mice were immunized once with IVT’s vaccine formulations and once or twice with an appropriate control vaccine. Serum was collected at week 2, 3, 4 and monthly thereafter and tested using an H5 ELISA. The therapeutic efficacy of cancer vaccine formulations was tested in an established tumor challenge model. Mice bearing C3 tumors were vaccinated once with IVT’s depot formulations containing the immunodominant CTL epitope from HPV16 E7. Tumor growth and survival was monitored for 6 weeks. Antigen specific CTL activity was detected by IFN-γ ELISPOT in the lymph nodes of HLA-A2/H2-D (AAD) transgenic mice immunized with HLA-A2 restricted CTL epitopes formulated in IVT’s depot vaccines.

Results: H5 formulated in our depot technology was able to raise a strong immune response within 18 days. At all time points tested, IVT depot formulation titers were superior to a single dose of the control alum vaccine and in the longer term superior or equal to the two dose alum vaccine. In the therapeutic C3 tumor challenge model, a depot formulation effectively eliminated C3 tumors after a single dose (100% tumor free mice) compared to non-vaccinated mice (0% tumor free, mean tumor size >2000 mm3). An HLA-A2 peptide-based therapeutic cancer vaccine has been designed for Breast/ Ovarian/ Prostate cancers and antigen specific immune responses were detected in AAD mice after a single dose.

Conclusions: 1) Single dose capability and 100% response rate of IVT’s depot vaccines are significant in the context of a pandemic vaccine for which low initial responses and overall low individual response rates could lead to many deaths. 2) The multi-targeting strategy using tumor-specific peptides and potent cellular response induced by IVT’s depot vaccines indicates a promising immunotherapy for cancer.

Comprehensive HIV/AIDS Care And Treatment As A Need For Quality Provision Of Antiretroviral Therapy: A Case Study From Dar Es Salaam Region, Tanzania

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Background: The roll-out of Tanzania National HIV/AIDS care and treatment program began in October 2004, with a plan target to cover about 400,000 HIV infected Tanzanians in a period of five years. In delivering Antiretroviral Therapy (ART) a certain level of quality is recommended. The objective of this study was to determine the quality standards of health facilities in providing HIV/AIDS care and treatment in line with the Ministry of Health (MOH) stipulated guidelines.

Methods: A cross-sectional descriptive study was conducted to assess the quality standards in delivering ART in Dar es Salaam region from May to July 2005. Ten health facilities (both public and private) already designated by MOH to provide ART, six of them since October 2004 (included purposively) and four since May 2005 (selected randomly). The other two facilities not designated were randomly picked and added. The checklist with the MOH required standards was used to assess the availability of equipments, staff, antiretrovirals, guidelines and adequacy of services provided.

Results: Regarding services provided, it was found that Comprehensive HIV/AIDS care and treatment was not fulfilled in all health facilities as recommended. More than half of the health facilities did not have Home Based Care (HBC) services. However, PASADA (Pastoral Activities and Services for people with AIDS in Dar es Salaam Archdiocese) with 14,000 patients, had a strong HBC with no patient lost to follow-up (The percentage of patient lost to follow-up ranged from 0% to 7.3%). Prevention of Mother to Child Transmission (PMTCT) services was found in two third of facilities. Although food support is included as an element of comprehensive HIV/AIDS care and treatment, only PASADA Voluntary Agency was providing nutrition services.

Figure: Percentage of health facilities with HIV care and treatment services

Conclusion: The advantage of comprehensive HIV/AIDS care and support services was shown by PASADA with the example of no patient lost to follow-up. The success shown by PASADA should be adopted by other health facilities for quality provision of ART.
Is Cannabidiol (CBD) An Accurate Marker For Cannabis Use? Analysis Of 2007-2008 Blood GC/MS Analysis From Drivers Under Influence

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Cannabidiol (CBD) is a psychoactive substance that is a constituent of cannabis. Most abstracts are listed in alphabetical order of the presenting author.

Cannabinoids are the natural constituents of cannabis. The main of them are delta-9-tetrahydrocannabinol (D9-THC), psychoactive agent; cannabidiol (CBD) and cannabichromene (CBD). A single active dose of D9-THC is estimated on 300 mg. mg/D9-THC is rapidly metabolised; it is hydroxylated to an active metabolite, 11-hydroxy-delta9-tetrahydro-cannabinol (11-OH-THC) in the liver, which is oxidised to an inactive 11-nor-carboxy-delta9-tetrahydro-cannabinol (THC-COOH), which is conjugated in the liver. The resulting metabolites are eliminated from the body. THC is rapidly distributed to blood pressure, which is not a common factor, and often common after a dose is taken that exceeds an individual variable threshold. The maximum effect period of THC is not related to the THC receptor density of the blood. THC plasma concentration declined to values of 2-3 ng/ml during 3-4 h after smoking. Such a low concentration of the active compound justifies the use of sensitive analytical methods for detection and determination of THC and its metabolites. The most effective techniques for THC and related compounds determination in biological material are chromatographic ones (gas and liquid) and mass spectrometric methods (GC-MS, LC-MS).

Extraction and quantification of active metabolites THC and 11-OH THC by GC/MS is currently used for samples collected by emergency forensic physicians from individuals suspected of drug use and involved in road traffic accidents. 11-OH THC is verified by analysis of blood by gas chromatography/mass spectrometry (GC/MS) technique. In 2007, by the emergency forensic physicians of the Compiegne hospital (Picardy, France) to cases of road traffic accidents. Quantification of metabolites was done by gas chromatography/mass spectrometry (GC/MS) technique. The results showed that the majority of the drivers sampled positive were regular users of cannabis or users of concentrated forms of cannabis.
Mannose-Binding Lectin In The Defence Against Genital Candida Infections

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Background: Mannose-binding lectin (MBL) is active in the innate immune defence against genital infections. MBL binds to C. albicans and is considered to protect against vuvulovaginal candidiasis (VVC) and such recurrent infections (RVVC). RVVC is not a chronic infection but characterized by repeated attacks. RVVC cases are often candida culture-negative in spite of symptoms and signs of vaginitis in between the attacks.

Material and Methods: Twenty-nine women with a history of RVVC were investigated, who had all consulted the Gynaecological Department at the University Hospital, Lund. The comparison group consisted of 30 women who were staff at the Department. All patients reported to a standardized history form and were subjected to a speculum investigation. Cultures of vulvar and vaginal samples collected from the lateral vaginal wall and the posterior fornx were grown on Chromagar, which allowed speciation of any candida isolate. Serum levels of MBL were determined by a sandwich time-resolved immunofluorometric assay, using anti-MBL coated microtiter wells containing, samples, which were washed and incubated with biotinylated anti-MBL followed by europium-labeled streptavidin and measured by time-resolved fluorometry.

Results: The serum levels of MBL in the RVVC group, age 21-58 (mean 31) years, ranged from <10 to 5120 ng/mL (mean 1580 ng/mL). The corresponding figures for the comparison group, age 21-64 (mean 41) years, were <10 to 2987 ng/mL (mean 920 ng/mL). The difference was significant (p=0.006). The levels were higher in culture-positive (44.8%) than culture-negative RVVC patients (p=0.003), and in repeated RVVC cases, but one (C. glabata) of the isolates. RVVC who were candida-negative when consulting had MBL levels comparable to the controls. There was no difference in MBL levels between women of different ages (mean 21-64 years, ranged from <10 to 5892 ng/mL (mean 1590 ng/mL). The corresponding figures for the comparison group, age 21-64 (mean 41) years, were <10 to 2987 ng/mL (mean 920 ng/mL).

Conclusions: MBL levels were higher in women with a history of RVVC than in the controls as well as higher in those who at samplings occasion were candida-positive compared with candida-negative women. MBL may have a therapeutic effect in cases of recurrent genital candida infections.
Can We Make Eucaryotic Cells Resistant To Antibiotics? Correlation Between Multidrug Resistance-Associated Protein (Mrp) Efflux Pump Expression And Fluoroquinolones Accumulation In J774 Macrophages

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Background: Over-expression of multidrug transporters is a well-known mechanism of resistance to anticancer agents in eucaryotic cells, and to antibiotics in immune cells. In vitro, these cells can be over-exposed to drug substrates. We have examined here whether chronic exposure to fluoroquinolones antibiotics could trigger a phenotype of resistance in eucaryotic cells as well, based on our previous observation that ciprofloxacin (CIP) is subject for an active efflux by an Mrp-like (Multidrug resistance-related Proteins) transporter in J774 macrophages (MBE), which decreases its accumulation and activity against intracellular bacteria, while moxifloxacin (MXF) was not affected (AAC 2005, 49:2429-2437).

Methods: Results: Conclusions: There are some indications of a statistically but not necessarily clinically significant advantage in antidepressant efficacy advantage of drugs enhancing neurotransmission of both serotonin and norepinephrine compared to SSRIs. There is no consistent evidence that SSRIs or SNRIs are superior in efficacy to older non-selective antidepressants. Newer non-selective antidepressant agents under development such as the triple uptake inhibitors hold promise. Similarly, studies support greater antidepressant efficacy with the broad spectrum agents such as clazapine compared to haloperidol in the treatment of schizophrenia.

Authors’ disclosure statement: Dr. Marks has received research support from Merck and National Institutes of Health and is on the speaker’s bureau of Eli Lilly and Company and Pfizer. Dr. Patkar is a consultant for Bristol-Myers Squibb, GlaxoSmithKline, and Reckitt Benckiser; has received research support from National Institutes of Health, AstraZenea, Bristol-Myers Squibb, Forest, GlaxoSmithKline, Janssen, McNeil Consumer and Specialty Inc, Organon, Jazz Pharmaceuticals, and Pfizer.

Success Story Of The First Regulatory Approval Of Safety Biomarkers, Part I: From Identification To Biological Qualification

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Background: In the context of the Critical Path Initiative, a project on the validation and qualification of new renal safety biomarkers has found a successful closure.

Methods: The project focused on rat nephropathy, whereby 8 known nephrotocics and 2 known hepatotocics were administered at different dose levels to assess diverse modes and severity grades of toxicity. To enrich the set of functional markers (serum creatinine and blood urea nitrogen) used for the last hundred years, gene expression profiling was performed in the kidney and correlated to the histopathological changes. This project also allowed the establishment and testing of the implementation of an early safety biomarker validation process. The first step of the process was the discovery phase and selection of exploratory biomarkers, model compounds and the definition of the animal model. Then, appropriate analytical methods (multiplex immunosassays and RT-PCR) were developed and validated. The in-life phase started with a dose-range finding study followed by the main validation studies comprising of 960 animals in total. In these multiple models of kidney injury, the sensitivity and specificity of the new markers were assessed and compared to the “gold standards” serum creatinine and blood urea nitrogen. In addition, a gene expression atlas across multiple organs in baseline and treated animals was compiled for the best performing markers.

Conclusions: A subset of these markers demonstrated higher diagnostic performance than serum creatinine and blood urea nitrogen, especially for low-grade tubular and glomerular injury. These data were submitted to the FDA and EMEA as first Voluntary eXpertatory Data Submission (VXDS) and subsequently 9 urinary biomarkers were approved for use as biomarker to monitor renal injury in preclinical studies.
**Background:** Stealth adaptation of viruses refers to the loss of the relatively few viral antigens that are normally targeted by the cellular immune system. Consequently, these viruses evade effective recognition by the cellular immune system. Steadily adapted viruses are postulated to be a major cause of human illnesses, especially those with prominent neuropsychiatric features, including autism in children and depression/cognitive disorders in adults. A non-immunological, auxiliary defense mechanism can repair the cytopathic effect (CPE) caused by stealth adapted viruses in tissue cultures. The repair is mediated by particulate, pigmented materials that are typically fluorescent, occasionally magnetic and can show both electron donating and water splitting capabilities. Ultraviolet (UV) light evoked fluorescence can commonly be enhanced using various dyes, including neutral red. The materials seemingly provide a non-mitochondria source of cellular energy. Alternative cellular energy (ACE) pigments are detectable in tissues and body fluids of patients with various illnesses. Comparable materials, termed Energeticals, are being formulated for potential clinical use in illnesses caused by both stealth adapted and conventional viruses.

**Methods:** In an ongoing study in patients with autism, paper towels moistened with a particular Energetical preparation and neutral red dye, are layered onto a polyethylene sheet that covers parts of the body. The paper towels are rendered fluorescent using UV-A illumination. The patients are observed for skin fluorescence occurring elsewhere on the body and for post-treatment signs of clinical improvement.

**Results:** Major clinical improvements, described and updated regularly at www.imirhere.ca, are occurring in autistic patients following 2-5 daily, 30-60 minute sessions using the above protocol. Single therapies are also achieving unexpected healing of active HSV infections and with HIV2 induced post-herpetic neuralgia.

**Conclusion:** Activation of the ACE pathway can provide an effective means of treating illnesses due to both stealth adapted and conventional virus infections.

**New Horizons In Respiratory Allergy Therapy And "Magic Bullets": Could It Be Possible To Include Antiprotozoal Drugs?**

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**Background:** The allergic diseases that affect the respiratory pathways have reached epidemic proportions in recent decades. For many years now, a close relationship has been established between these diseases and products in the therapy of patients with recurrent herpetic simplex (HSV) infections and with HIV2 induced post-herpetic neuralgia.

**Methods:** To review the publications available in different fields of medicine that refers unknown and unknown kinds of protozoa that may affect the human airways.The literature review was identified through electronic data bases such as MEDLINE, EMBASE, and the COCHRANE DATABASE OF SYSTEMATIC REVIEWS. Peer-reviewed publications in English, French, and Spanish language, and English-language abstracts of non-English papers, identified in our research, were included.

**Results:** Uncommon multi-flagellated protozoa belonging primarily to Phylum Sarcomastigophora, Order Hypnastigida, and observed in intestinal extracts of mites and cockroaches, also have been found in human respiratory airways secretions, especially in patients with respiratory allergy (bronchial asthma and allergic rhinitis) and/or immunosuppression status (AIDS, transplants, cancer, etc.).

**Methods:** To review the publications available in different fields of medicine that refers unknown and unknown kinds of protozoa that may affect the human airways.

**Results:** Uncommon multi-flagellated protozoa belonging primarily to Phylum Sarcomastigophora, Order Hypnastigida, and observed in intestinal extracts of mites and cockroaches, also have been found in human respiratory airways secretions, especially in patients with respiratory allergy (bronchial asthma and allergic rhinitis) and/or immunosuppression status (AIDS, transplants, cancer, etc.).

**Conclusion:** It is evident that, despite great efforts and new therapeutic approaches, allergic respiratory diseases continue to be on the increase. Until now, only the possible etiopathogenic role in the development of respiratory allergy. This role may be reinforced if we take into account that the professional method acquires during the teaching-learning process a greater importance than answering a specific problem. The scientific facts and data learnt today can become tomorrow’s accepted or even rejected in a near future. On the contrary, those pharmacists who can identify and solve their patient's drugs - related - problems by applying a reasonable methodology and/or immunosuppression status (AIDS, transplants, cancer, etc.).

**Conclusion:** Activation of the ACE pathway can provide an effective means of treating illnesses due to both stealth adapted and conventional virus infections.

**Optimizing A Therapeutic With Nsaids: Intelligent Design For Delivery Systems**

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**Background:** There is a wide approach to modulate drug release with the objective of optimizing therapy. The type of drug will define the type of release that is to be achieved. The NSAIDs are used in anti-inflammatory diseases, so it would be desirable to develop a quick/slow delivery system to alleviate the painful symptoms and to avoid repeated hospital visits. A flexible release delivery systems based on compressed or encapsulated mini-tablets 2) To study the dosage regimen flexibility. 3) To study the dosage regimen flexibility. 4) To prolonged-release component. The mini-tablets contained either HPMC or EC as controlling agents and Ibuprofen as a model drug. Mini-tablets, ibuprofen loading of 1 mg and 2.5 mm, were prepared by direct compression in an instrumented mechanical press machine. Fast-release component. The filling of the void spaces between mini-tablets was formulated to produce an immediate release. The composition was identical for all formulations and contained the immediate release drug dose, microcrystalline cellulose and sodium croscarmellose. Encapsulated mini-tablets system. The die of the tabletting machine was progressively filled with the weighed amounts of the fast release component and mini-tablets prior to compression. Tablets were prepared by direct compression. Encapsulated mini-tablets systems: These systems were prepared by encapsulating the weighed amounts of the fast release component and mini-tablets in a hard capsule. The dosage regimen flexibility was studied by combination of a different number of mini-tablets (prolonged release component) and a different dose of the drug (fast release component).

**Results:** The biphasic delivery systems were characterized by an initial rapid release, corresponding to the drug release contained in the powder component, followed by a period of slow release, corresponding to the drug release of mini-tablets. The release profile was dependent on the number and/or composition of mini-tablets, making up the drug sustained dose. After the deintegration of the biphasic systems, the HPMC subunits were able to release a second dose fraction in a prolonged time (~7h) at a constant rate and with an identical dissolution profile to the original mini-tablets. In the case of biodegradable EC mini-tablets systems, the releasing of fast component disturbed the drug diffusion mechanism.

**Conclusions:** 1) Bicentric slow/rapid preparations of ibuprofen were being developed by compressing or encapsulating a combination of powder and mini-tablets. 2) The proposed biphasic delivery devices show flexibility in the modulation of the delivery program.
Blood withdraws from a rock in order to quench a sick girl's thirst” (“The Nobel Prize Medals”).

The Nobel Prize in Physiology or Medicine is awarded once a year by the Swedish Karolinska Institute. It is one of the five Nobel Prizes established by the will of Alfred Nobel in 1895, awarded for outstanding contributions in physics, chemistry, literature, peace, and physiology or medicine since 1901.

The first Nobel Prize in Physiology or Medicine was awarded in 1901 to Emil Adolf von Behring, a German, “for his work on serum therapy, especially its application against diphtheria, by which he has opened a new road in the domain of medical science and thereby placed in the hands of the physician a victorious weapon against illness and deaths.” This award is administered by the Nobel Foundation and widely regarded as the most prestigious award that a scientist can receive in these fields.

It is presented in Stockholm at an annual ceremony on December 10, the anniversary of Nobel’s death. “The highlight of the Nobel Prize Award Ceremony in Stockholm is when each Nobel Laureate steps forward to receive the prize from the hands of His Majesty the King of Sweden. … Under the eyes of a watching world, the Nobel Laureate receives three things: a diploma, a medal and a document confirming the prize amount” (“What the Nobel Laureates Receive”). In 2007 the Nobel Prize in Physiology or Medicine was awarded to Mario Capecchi (of Italy), Sir Martin Evans (of the United Kingdom), and Oliver Smithies (of the United Kingdom and the United States), “for their discoveries for introducing specific gene modifications in mice by the use of embryonic stem cells”; they share the prize amount of 10,000,000 SEK (slightly more than €1 million, or US$1.4 million). The front of the medal on the back side “represents the Genius of Medicine holding an open book in her lap, collecting the water pouring out from a rock in order to quench a sick girl’s thirst” (“The Nobel Prize Medals”).

Effect Of Formulations On Clopidogrel Bioactivity And Bioavailability In Vivo

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Clopidogrel hydrogen sulphate is antiplatelet agent approved for use in secondary prevention of heart attacks and stroke. Although millions of cardiac patients are benefiting from Clopidogrel treatment, however, nearly 5% of the patients are Clopidogrel’s “non-responders.”

The pharmacokinetics parameters of Clopidogrel, under fasting conditions, are conflicting as to the maximum concentration achieved following the dose (Cmax) with a range of 1.2 ng/ml to 9 ng/ml and a time to maximum concentration (Tmax) of 1 hour to 2.5 hours.

These results are more controversial under fed conditions with studies showing no effect of food on the pharmacokinetics of Clopidogrel in Caucasians while studies in the Asian population demonstrated a significant influence of food. In these studies Tmax increased from 2.5 hours to 5 hours and the Cmax was increased by 5 folds. Moreover, there is a direct effect of genetics on the absorption and thereby active metabolite formation which are diminished by P-gp.

Further advances in the instumentation of ESR imaging and would make this technology even more promising for the non-invasive diagnosis of oxidative stress induced-brain diseases in vivo. Furthermore, after screening test of drugs or foods using in vivo ESR technique, we’ll be able to develop and find drugs or foods with novel antioxidant property in the near future.

Integrated "OMIC" Analyses Of The Rat Brain: Novel Biomarker Candidates For Mental Disorders And Stress

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Background: It has been well known that stress may cause mental disorders. However, the process from stress to disorders is not clear yet. Aims: 1) To clarify the effects of several kinds of stress on the brain. 2) To understand the key role of animal models for mental disorders, such as depression and developmental disorders. 3) To determine multiple stress markers that may play important roles in mental disorders.

Methods: This study included 280 rats. We prepared animal models for attention-deficit hyperactivity disorder (ADHD) and depression. For ADHD, male rats at 5 days of age received intracranial injection of 6-hydroxydopamine (6-OHDA) or environmental chemicals. Male congenic wigging (Wig) rats were generated using Long-Evans and Wistar strains. For depression, adult male rats were exposed to the stress, such as immobility or water. Moreover, we examined the effects of continuous light, gamma knife treatment and alcohol drinking. These brains were analyzed by transcriptomics using DNA microarray and proteomics with two-dimensional gel electrophoresis followed by mass spectrometry. In some experiments, we performed metabolomics by NMR.

Results: A deficit in the development of dopamine (DA) neurons caused behavioral hyperactivity similarly to ADHD. Alterations in the expression of genes and proteins in brain regions showed variation among environmental chemicals and differed from those of 6-OHDA-injected and Wig rats. With these techniques, we found stress marker candidates that were similarly altered by immobility, water, and continuous light. Among these, we observed that coffee bean aroma attenuated the effects of water stress (sleep deprivation). Moreover, several stress marker candidates were found after gamma knife treatment and alcohol drinking.

Conclusions: 1. Deficient development of DA neurons may underlie motor hyperactivity, and additional factors may be altered in Wig rats and animals exposed to environmental chemicals, which may reflect different types of ADHD patients. 2. Different kinds of stress that may cause depression altered similar potential biomarkers in the brain. 3.OMIC tools will be useful to study possible alterations in the expression of multiple factors in the brain.
Inhibition Of Tumor Metastasis And Angiogenesis By NK4, Bisubtilic Inhibitor Of HGF-Met And Angiogenesis

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Background: Hepatocyte growth factor (HGF) and the Met/HGF receptor tyrosine kinase play a crucial role in invasion and metastasis of a variety of cancer cells. HGF induces dissociation of cell-cell contact, breakdown of extracellular matrix, and concomitant migration of cells, thereby potentially enhancing cancer invasion and metastasis. Thus, HGF-Met system is a notable molecular target in cancer therapy. We recently reported a sizable anti-angiogenic activity of NK4.

Methods: We prepared NK4, a competitive antagonist for HGF-Met. NK4 is an internal fragment of HGF that encompassing the N-terminal and subsequent four amino acid residues.

Results: We found that NK4 acts as an angiogenesis inhibitor as well as HGF-antagonist. NK4 inhibits angiogenesis driven by vascular endothelial cell growth factor and basic fibroblast growth factor, as well as HGF. Through its binding to a protein different from Met receptor, NK4 inhibits cell migration in induced by vascular endothelial growth factor (VEGF) was also suppressed by vitamin K at 5 s. Vitamin K selectively inhibited DNA polymerase , however vitamin K and K2 had no effect on DNA polymerase activity.

Conclusions: 1) NK4 exerted anti-angiogenic activity through inhibiting important angiogenesis processes. 2) NK4 selectively inhibited DNA polymerase activity, however vitamin K, which has anti-angiogenic activity, had no effect. 3) Vitamin K2 could be a potent anti-cancer agent.

The Effects Of Single-Dose Ethanol Administration To Aldehyde Dehydrogenase 2 Knock-Out Mice: Down-Regulation Of Expression Of Cytochrome P450 2E1 Mrna And Amelioration Of Oxidative Stress In Liver Tissue

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Background: The polymorphism of aldehyde dehydrogenase 2 (ALDH2), denoted ALDH2*2, is very common in East Asian people. Acetaldehyde, an intermediate metabolite of ethanol, is metabolized very slowly in people with ALDH2*2 because the mutant ALDH2 protein lacks the activity of acetaldehyde metabolism. On the other hand, one of the cytochrome P450 enzymes, CYP2E1, is well known as an activator of carcinogens and a generator of oxidative stress, and CYP2E1 is induced by ethanol via gene transcriptional regulation. Oxidative stress is also generated when ethanol is metabolized with enzymes other than CYP2E1.

Methods: To examine the consequences of the ALDH2*2 polymorphism, we examined CYP2E1+ and −/− mice which were orally administered ethanol at a dose of 5 g/kg body weight and the levels in liver tissue of CYP2E1 mRNA, malondialdehyde (MDA, an indicator of oxidative stress), and glutathione (GSH, a key antioxidant), were then analyzed 0-72 hours after administration.

Results: The level of CYP2E1 mRNA 12 hours after ethanol administration in ALDH2+/mice was significantly lower than that in the 0 hour ALDH2+/− group and that in the 12 hour ALDH2+/− group (*, p < 0.05). The levels of MDA were significantly lower in ALDH2+/− mice than in ALDH2+/+ mice at 12 hours after ethanol administration, while levels of GSH were significantly higher in ALDH2+/− mice than in ALDH2+/+ mice at 6 and 12 hours after administration.

Conclusions: 1) Single-dose ethanol administration down-regulates the expression of cytochrome p450 2E1 mRNA in the presence of inactive ALDH2. 2) A lack of ALDH2 ameliorates ethanol-induced oxidative stress in liver tissue.
Background: Metachromasy (metachromasia) is a well-known observation in biological and medical experiments. This phenomenon often allows to discriminate a certain substrate or tissue from others and thus metachromatic staining has been used as a cytological and diagnostic tool. Metachromatic colour changes have been considered as being caused by dye aggregation. However, the structure of the dye aggregates is still unknown. In this study, as a first step for our goal to understand structure-function relationships of biomolecules by means of metachromatic staining, the metachromatic spectral changes of Toluidine blue(TB) were considered by computational approach.

Methods: TB-DNA complex models were constructed by the optimized molecular structure of TB calculated by quantum calculations (Software: CAChe ver 5.2, Hamiltonian: AM1 for structure optimization and ZINDO/S for electronic properties) and crystal structures of DNAs obtained from Protein Data Bank (B-DNA: 1BN A and A-DNA: 440D). The dimethyl amino group of TB was assumed to bind to a phosphate group of DNAs by electrostatic interaction. Displacement energies for the TB aggregates were estimated by the extended-dipole model using the calculated transition dipole moment of TB corresponding to its visible properties and crystal structures of DNAs obtained from Protein Data Bank (B-DNA: 1BN A and A-DNA: 440D). The dimethyl amino group of TB was assumed to bind to a phosphate group of DNAs by electrostatic interaction. Displacement energies for the TB aggregates were estimated by the extended-dipole model using the calculated transition dipole moment of TB corresponding to its visible properties and crystal structures of DNAs obtained from Protein Data Bank (B-DNA: 1BN A and A-DNA: 440D). The dimethyl amino group of TB was assumed to bind to a phosphate group of DNAs by electrostatic interaction. Displacement energies for the TB aggregates were estimated by the extended-dipole model using the calculated transition dipole moment of TB corresponding to its visible properties and crystal structures of DNAs obtained from Protein Data Bank (B-DNA: 1BN A and A-DNA: 440D).

Results: In TB-DNA complexation, the two adjacent dye molecules gave a hypochromic spectral shift in the range from 117 to 330 cm⁻¹. The resulting spectral shifts were strongly related with the geometry of DNA. The twist angle and the alpha-angle were found to play an important role in the displacement energy. A small difference between the alpha-angles of adjacent phosphate groups resulted in a parallel arrangement of TB molecules forming a large spectral shift. In TB-A-DNA complexation, on the other hand, the steric restriction in the tightly rolled A-DNA sterically restricted the highly tilted A-DNA.

Conclusions: Some of the author's current interests and recent progress on these problems will be described.

IM28 Inhibiting HIV1 Replication, Glucose, Lipids, Hemoglobin Levels And Nitric Oxide (NO) in HIV1 Patients: Study From Gabon

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Background: IM28, an analog of dehydroepiandrosterone (DHEA) demonstrates deeply anti-viral activity including, the inhibition of the reverse transcriptase activity, the restriction of envelop proteins mediating cell-cell fusion (gp120) and the dimer formation. A small difference between the alpha-angles of adjacent phosphate groups resulted in a parallel arrangement of TB molecules forming a large spectral shift. In TB-A-DNA complexation, on the other hand, the steric restriction in the tightly rolled A-DNA sterically restricted the highly tilted A-DNA.

Methods: In the composition of TB with the B-DNA, hypochromic TB dimers were found to form with the DNA. Their spectral shifts are strongly influenced by the twist angle and the alpha-angle. 2) The dimer formation is sterically restricted in the tightly rolled A-DNA.

Results: No side effects attributable to IM28 were noticed regarding hepatic, cardiac and renal functions evaluation as no significant difference was seen with baseline of urea, creatinine, GGT and TGP. By contrast to patients treated with DHEA, normalization of glycaemia, increased body weight, CD4 (+0.01), lymphocytes and hemoglobin levels (+0.001) parallelized by significant reduction of platelets, antigenemia p<24 (p<0.001) and viral load (p<0.01) were observed in patients under IM28 treatment. In addition, IM28 normalized body weight, lipids, glucose levels and blood pressure in obese, hypertensive and diabetes patients more to reduce significantly the percentage of opportunistic afections such as tuberculosis, malaria, deep tissue, urinary tract, stroke, skin rashes, digestive tract, urinary tract, stroke, facial paralysis, language and memory troubles, dementia and anxiety. Moreover, the body temperature which is always higher in HIV1 patients and persisting under HAART treatment was reduced and normalised in patients under IM28.

Conclusion: Data suggest that, the use or the substitution of DHEA which is already used as supplement of HIV1 treatment by IM28 should represent a better therapeutic avenue for HIV-1 and opportunistic related diseases as well as for cardiovascular diseases. Magic bullet effects of IM28 may be partly due by nitric oxide (NO) through the normalisation of his reservoir haemoglobin levels.

A Concerted Approach Using Physical Chemical Methodologies, Computational Chemistry And 3D QSAR Studies Aiming To Develop Novel Analogic Cannabinoid Analogs

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The C-1'-dihydrolinole 5',6'-tetrahydrocannabinol (5',6'-THC) amphiphilic analogue (–)-2-s, 6a,7,10,11,12-hexahydro-6,6,9-trimethylhydroxy-6H-dibenzo[b,d]pyran)[2]-hexyl-1,3,4-dioxolane (AMG3) is considered as one of the most potent synthetic anergic cannabinoids (CB2) ligands. Its structure is characterized by a rigid bicyclic and flexible alkyl chain segments. Its conformational and ADME properties have been explored using a combination of physical chemical methodologies, computational chemistry and 3D QSAR studies. More particularly, a strategy is developed in which its conformational properties are studied in an ascending complexity. Thus, the conformational properties of AMG 3 are explored in vacuum, in amphoteric solvents, in the receptor site and in a bilayer environment that closely simulates the biological one. Two sites are discovered in the binding pocket (see Figure below ) which show distinct conformational preferences and structural requirements (S1 specific and S2 specific). These sites can be explored for a future design and may constitute a magic bullet target for potential anergic drugs. Two examples of potential magic bullet targets are given below.

All abstracts are listed in alphabetical order of the presenting author.
Amifostine (WR-2721) As A Cytoprotective Agent

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Background: Chemotherapy and radiation therapy are the basic approaches in cancer treatment. The current therapies are associated with significant cytotoxicity, as chemo- and radio-therapy do not discriminate between normal and pathological cells. The attempt to increase the therapeutic ratio in an effort to improve survival or quality of life is the goal of modern cancer therapy. The use of cytoprotective agents is one approach to minimizing the toxicity caused by the therapy to normal cells. Aims: 1) to review the vast literature published on amifostine, the aminothiol formerly known as WR-2721, 2) to demonstrate the results of the preclinical experiments and clinical experience with amifostine, 3) to summarize the knowledge on chemo- and radio-protective effects of WR-2721 on normal cells, 4) to show the effects of the aminothiol on programmed cell death, 5) to determine the mechanisms of cytoprotection induced by amifostine, and 6) to provide the insight into future experimental and clinical directions.

Methods: A review of the data bases on a collection of the original papers published and the abstracts of relevant articles searched in the SCOPUS and MEDLINE database.

Results: Amifostine was developed to selectively protect various normal tissues against the hematopoietic, nephro-, neuro-, and cardio-toxicity, mucositis and xerostomia caused by ionizing radiation and/or chemotherapeutic drugs, e.g. platinum agents, oxazaphosphorines, anthracyclines, taxanes. Amifostine provides a broad-spectrum of cytoprotection against the radio- and chemo-toxicity observed in patients with breast, bladder, cervix, head and neck, non-small cell lung cancer. There is evidence that amifostine, alone or in combination with other chemotherapeutic agents, is effective in prolonging the survival of patients with breast and bladder cancer. Amifostine has potential applications in many oncologic settings, but the precise mechanisms whereby WR-2721 exerts the cytoprotective action on the cancer cells are not entirely clear. A better understanding of the mechanisms responsible for the cytoprotective effects of Amifostine can contribute to the further development of this agent.

Conclusions: 1) After several decades of preclinical and clinical research, amifostine is widely used in clinical practice as the best known cytoprotector against the adverse effects of chemo- and radiotherapy, 2) As a chemo- and radio-protector of normal cells, amifostine is accepted to be a powerful adjuvant to the current therapies.
Background: The aim of the study was to determine the effect of newer NSAIDs, Proton Pump Inhibitor, artemisinin comb wha pharmacokinetics of phenytoin and carbamazepine levels were assessed by HPLC, and pharmacokinetic parameters were calculated.

Results: Treatment with etoricoxib and acceclofenac, there was a decrease in t1/2(2) and t1/2(1) significantly as compared to phenytoin and carbamazepine group alone. Significant changes were observed in the pharmacokinetic parameters in etoricoxib and acceclofenac treated group. With esomeprazole and co-administered with artemisinin, artemether or arteether. The increase in plasma phenytoin and carbamazepine levels was assessed by HPLC, and pharmacokinetic parameters were calculated.

Methods: In a parallel design study, phenytoin (30 mg/kg/day) and carbamazepine (40 mg/kg/day) orally were given for daily 7 days in 12 rabbits. On day 7 and 14 day, blood samples were taken at various time intervals between 0 and 24 h. Animals were treated with etoricoxib, acceclofenac, esomeprazole, rabeprazole, artemisinin, artemether, and arteether from 7 day onwards to 14th day. Plasma phenytoin and carbamazepine levels were assessed by HPLC, and pharmacokinetic parameters were calculated.

Conclusion(s): These results suggest that newer NSAIDs, Proton Pump Inhibitor, was co-administered with artemisinin, artemether or arteether. The increase in plasma phenytoin and carbamazepine levels was assessed by HPLC, and pharmacokinetic parameters were calculated.

Effect Of Commonly Prescribed NSAIDs, Proton Pump Inhibitor And Newer Anti-Malarial Compound On Pharmacokinetics Of Different Antileptepics

Transgenic Mice

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Background: Immunocontraception would be dramatically broadened to more recipients if direct 'off-the-shelf' products could be engineered to engender functionally potent immune responses against true self tumor antigens. The carcinoembryonic antigen (CEA) is upregulated in colon cancers, non-small cell lung cancers, and breast cancers. CEA is an excellent and important target for cancer immunotherapy.

Methods: The human CEA CDNA was used to construct the lentiviral vector (LV-CEA). huCEA Tg mice were used for anti-tumor immunity studies. A murine gastric carcinoma cell line expressing huCEA was used to establish subcutaneous tumors. Mice were grafted at day 0 with 5.8-10^7 murine CRC246 colon cancer cells in the flank.

Results: We show stabilization (mostly long-lasting) of 14-day established subcutaneous m6G4CEA tumors in human CEA-transgenic mice following two direct low-dose injections of LV-huCEA and not LV-ENFP. This stabilization result was reproducible and detailed analyses including antibody assays, multiplex cytokine analyses on unstimulated splenocytes, tetramer staining, and immunofluorescence staining of tumor sections demonstrated that this outcome correlated with both a cellular and humoral immune response.

Conclusions: We observed that we can safely break tolerance to huCEA and engineer an efficient anti-tumor response along with anti-huCEA antibody and CTL responses. These data support the use of direct injections of low doses of LV-huCEA for enhancement of tumor immunotherapy directed against CEA.
Antiviral Effect Of Lamivudine, Emtricitabine, Adefovir Dipivoxil, And Tenofovir Disoproxil Fumarate, Administered Orally Alone And In Combination, To Woodchucks With Chronic Woodchuck Hepatitis Virus Infection

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Adefovir dipivoxil (ADV) and tenofovir disoproxil fumarate (TDF) are nucleotide analogs that inhibit replication of wild-type hepatitis B virus (HBV) and lamivudine- (3TC) resistant HBV in patients, including patients co-infected with the human immunodeficiency virus (HIV). Combination of ADV or TDF with other nucleoside analogs is a proposed strategy for managing antiviral drug resistance during treatment of chronic HBV infection. The antiviral effect of oral administration of ADV (15 mg/kg/day) or TDF (15 mg/kg/day), alone or in combination with 3TC (15 mg/kg/day) or emtricitabine (FTC, 15 mg/kg/day) against chronic woodchuck hepatitis virus (WHV) infection was evaluated in groups of 5 woodchucks for 48 weeks. Dosages were chosen due to their efficacy in previous treatment studies in woodchucks. Once-daily treatment with the combination of ADV+3TC (4 survivors) or of TDF+FTC (4 survivors) significantly reduced serum WHV viremia from pretreatment level by 6.2 and 6.1 log10, respectively, as determined by dot-blot hybridization and real-time PCR (P<0.01). Additional findings include treatment with TDF+3TC (4 survivors, 5.6 log10), ADV (4 survivors, 4.8 log10), ADV+FTC (4 survivors, 4.4 log10), TDF (3 survivors, 2.9 log10), and FTC (5 survivors, 2.7 log10). Individual woodchucks across treatment groups had sustained effects. No signs of toxicity were observed for any of the drugs and drug combinations administered. In conclusion, oral administration of 3TC, FTC, ADV, and TDF, alone and in combination was safe and effective in the woodchuck model of chronic HBV infection.

Arenic Trioxide Induces Apoptosis Preferentially In B-CellL Cells Of Patients With Unfavorable Prognostic Factors Including Del17p13


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In the last decade, arenic trioxide has been used very successfully to treat acute promyelocytic leukemia (APL). Much less is known about the effectiveness of arenic trioxide in other neoplastic disorders. We report here that after 18 h in vitro treatment with 4 μM arenic trioxide, 75 ± 18 % of B-ALL cells (n = 52) underwent apoptosis. Importantly, B-ALL cells harboring a deletion of chromosome 17p13, which predisposes to fludarabine resistance and has been identified as an important negative predictor of clinical outcome, were more susceptible to arenic trioxide toxicity than cells lacking this aberration. Furthermore, unfavorable risk profiles such as unmutated IgVH status, high CD38 expression and prior treatment were associated with significantly higher sensitivity of B-CLL cells to arenic trioxide. Arenic trioxide also preferentially killed B-CLL cells as compared to B-cells from healthy age-matched controls. Molecular analysis revealed that basal superoxide dismutase activity was positively correlated with the pro-apoptotic activity of arenic trioxide pointing to a role of reactive oxygen species in cell death induction. The high activity of arenic trioxide in B-CLL cells from patients with unfavorable risk profiles indicates it a promising drug for high-risk and/or fludarabine-refractory B-CLL patients.

Gene Expression In Brain And Kidney In Response To Aluminium In Drugs

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Background: Aluminum (Al) has been used in various drugs such as analgesics, antacids and vaccines as well as the domestic uses. It has been believed that the absorption rate of Al from the intestine is quite low and the absorbed Al is excreted rapidly into urine. However, it is not known whether Al remains in the body in the case of the intake of Al-containing drugs. Al has been linked to several diseases as dialysis encephalopathy, osteomalacia, amyloidosis, amyotrophic lateral sclerosis and parkinsonisms-dementia in the Kii region in Japan. Al remains for a long period and circulates in the body. Even at a low concentration of Al, it may be a cause of essential hypertension due to the up-regulation of renin.

Psychoncology And Psychoneuroendocrinoimmunomune (PNEI) Status Of Cancer Patients

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It is known that the psychosomatic status may influence tumour growth and the prognosis of the cancer. Only recently a research group in PNEI discovered it a promising tool to characterize the neuroimmunochemical mechanism responsible for the influence of the psychosocial condition on tumour growth, through the modulation of the immune system. The anticancer immunome is stimulate by IL-2 and IL-12 whereas it is inhibited by IL-10 and IL-6. It has been observed that the evidence of low of IL-6 an IL-10, low number of lymphocytes and T-helper lymphocytes and an increase number of T reg is associated with the poor prognosis. Within the great number of the psychological variable, the spirituality has be considered as different from the psychological profile. According to the PNEI discoveries we have performed several studies to investigate the psychological profile of cancer patients in relation to their psychoneuroendocrinoimmunomune status. The psychological profile was investigated by the Rorschach's test and the spiritual status was analyzed by special spiritual test. We are performed five main studies, by obtaining the following results: 1) low number of lymphocytes and T-helper lymphocytes in patients with suppression of spiritual and sexual sensitivity; 2) Low efficacy of IL-2 immuno therapy and low lymphocytes response to IL-2 in patients with anxiety and or loss of sexual identity; 3) low number of lymphocytes associated with alteration in the circadian rhythm of cortisol and hypocortisolism in patients with low spiritual profile; 4) abnormal high percent of T- regulatory lymphocytes in patients showing self punishment status; 5) Lack of surgery induced-hyperprolactinemia in open breast cancer patients with suppression of the maternal behaviour. This results would suggest the possibility to investigate the psychoneurooimmunomune basis of the overall psychological profile in cancer patients. Further studies by investigating the brain endocannabinoid system through the detection of the blood concentration of the main endocannabinoid agent anandamide will clarify the neurochemical alteration responsible for the progressive loss of the pleasure in neoplastic disease.
Anticancer Gold Compounds: Mechanistic Insights

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Background: Gold compounds are a class of metallo-drugs, of potential great interest for cancer treatment. During the past two decades a large variety of gold(III) compounds were shown to manifest relevant antiproliferative properties in vitro against selected human tumor cell lines qualifying themselves as excellent candidates for further pharmacological testing. On turn, mechanistic studies pointed out that the interactions of cytotoxic gold compounds with DNA are generally far weaker than those of platinum drugs implying a different mode of action. The main goal of our investigation is to provide new insight into the possible mechanisms of action of this variegate family of cytotoxic drugs.

Methods: A variety of methods were employed to disclose the mode of action of gold drugs. Several gold compounds were prepared according to classical methods of coordination chemistry and extensively characterized in the solid state and in solution. Their biological properties were evaluated both at the molecular and the cellular level. Their effects on selected protein targets were investigated. In vitro cytotoxic properties toward a wide panel of human tumor cell lines were measured.

Results: A certain number of gold compounds, in most cases in the oxidation state +3, were prepared and characterised that manifested very pronounced cytotoxic effects in vitro. For most of them potent inhibition of thiorodoxin reductase was established. It is proposed that thiorodoxin reductase inhibition triggers cell apoptosis through a mitochondrial pathway. Moreover, extensive data have been collected on the cellular effects in vitro of a panel of gold compounds and specific insight into their respective mechanism of action has been achieved.

Conclusions: Gold(III) compounds constitute a novel family of cytotoxic drugs of great interest as potential anticancer agents. Their cytotoxic effects are mediated by a variety of molecular mechanisms that are deeply different from those of platinum compounds.

Bone Marrow Stromal Cells Attenuate Sepsis And Sepsis-Induced Acute Kidney Injury (AKI) Via A Novel Mechanism Of Action

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Background: Acute kidney injury (AKI) in septic shock is associated with a high mortality in ICU patients. Bone marrow stromal cells (BMSCs), which are so-called mesenchymal stem cells, have been shown to improve outcome in several different animal injury models by altering inflammation, apoptosis, and necrosis. In this study we set out to determine whether BMSCs can attenuate the severity of sepsis-induced AKI.

Methods: Sepsis was induced in C57BL/6 mice using cecal ligation and puncture (CLP). BMSCs were obtained from the bone marrow of 6-8 week old mice. Intravenous administration of BMSCs (10^6 cells/mouse) immediately before CLP surgery resulted in significantly longer survival and improved kidney, liver and pancreatic function. Such organ protection was not obtained with either hematopoietic stem cells or necrotic BMSCs. Searching for a mechanism of action we found that 24 hrs after CLP the serum levels of proinflammatory cytokines (TNF-, IL-6), peritoneal and kidney vascular permeability, splenic apoptosis and necrosis were significantly reduced in BMSC-treated animals vs controls; the level of IL-10, an anti-inflammatory cytokine, was not affected. Six hours after their injection, fluorescently-labeled BMSCs were detected mostly in the lung. By prelabeling BMSCs with quantum dots and later performing immunostaining with a macrophage marker (baa-1) we found the BMSCs adjacent to macrophages in the lung. Some were in the spleen, and rare cells were seen in the kidney; 24 hours after they were injected, we found few BMSCs in any organ. The positive effect of BMSC treatment was still present in Rag-/- mice; in NK cell depleted mice, and in IFN-/- mice, suggesting that both B and NK cells, and IFN- do not play a significant role in mediating the effects of the BMSCs. On the other hand, macrophage depletion or pretreatment with IL-10 or IL-10 receptor antibodies eliminated the beneficial effects of BMSCs in the sepsis model.

Conclusion: Our results suggest that BMSCs may act on tissue macrophages resulting in enhanced production of IL-10, a potent anti-inflammatory cytokine.

Novel Antineoplastic Agent – Peptide Conjugates As Drug Delivery Systems For Targeted Chemotherapy

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Background: Receptor-mediated tumor-selective drug targeting by peptides might be an efficient tool in cancer therapy. The combination of appropriate drugs with a targeting moiety that recognizes tumor-specific or overexpressed receptors on tumor cells might lead to more specific antineoplastic agents.

Aims: 1) To develop new conjugates containing antineoplastic agents and peptides with own biological activity. 2) To study the structure-activity relationship in vitro. 3) To optimize the in vivo treatment of the selected compound.

Methods: Flow cytometry was used with FITC-labeled annexin-V and PI stain, to discriminate intact cells from apoptotic and necrotic cells (Monotuftin) after treatment with the oligothiustin-Mtx conjugates and Mtx (10^3-10^5 M). In vitro antitumor activity was studied by MTT assay on MCF-7 human breast and C26 murine colon carcinoma cell lines. Treatments (Lp.) of 5-7 mcg/mg with free Daunorubicine (1x5 mg/kg or 5x5 mg/kg) or Dau-GnRH-III oxide conjugate (1x5 mg/kg or 5x5 mg/kg) were carried out on day 7 followed by repetition every second day in case of lower doses. The conjugate was applied in 1x5 mg/kg on days 4 and 7 and 7 after tumor transplantation.

Results: 1) Methotrexate-conjugate had cytotoxic effect. 2) Antitumor compound-GnRH-III conjugates with different linkages had the following IC50 values (M): ester (0.8) / hydrzone (1.5) / oxime (3,9) / amidole (100) on MCF-7, cells, which somewhat higher on C26 cell line. 2) Dau-GnRH-III oxide conjugate had antitumor activity and it was not toxic up to 15 mg Dau content/kg body weight. The best result was ~50% tumor growth inhibition and 33% increase of survival time to the control on C26 tumor bearing mice in case of treatments on days 4 and 7 after tumor transplantation.

Conclusions: 1) Efficient drug delivery into monocytes and macrophages was obtained by the application of tuftsin-like carriers. 2) The type of ligand had an influence on the antitumor activity. 3) Dau-GnRH-III oxide conjugate prevented the toxic side effect of Dau even at a concentration higher than the lethal dose of the drug. Significant decrease of the tumor growth and increased survival time was determined in case of treatments with Dau-GnRH-III.
Cancer Through The Right Bullets And Targets

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Functioning of the estrogen receptor (ER) is a prerequisite for outgrowth of hormone-dependent breast cancer. Anti-estrogens such as tamoxifen, inhibit ER function by inducing a structural alteration of the ER, which results in a reduced interaction with the transcription factor complex, ultimately leading to cell death. We have found that modification of the ERs by phosphorylation prevents the structural alterations that are the result of binding of anti-estrogens to ERs. This converts the anti-estrogen tamoxifen into an agonist. Different anti-estrogen demands for its specific modification(s) to render ERs resistant, providing a resistance "signature" (the target) for each anti-estrogen (the bullet). We generated an antibody that detects one of these modifications; the ERs phosphorylated at Serine 305 by Protein kinase A, and could identify resistance to tamoxifen in a group of breast cancer patients. Modifications in ER by phosphorylation provide a diagnostic tool for personalized breast cancer treatment, since alternative anti-estrogens are at hand for the signature-positive subgroup. They also guide optimal development of novel anti-estrogens. Supported by TIPharma and KWF.

Abstracts
All abstracts are listed in alphabetical order of the presenting author.

Abstracts
Page A-210
Blockade Of IL-6 Signalling With A Humanized Anti-IL-6 Receptor Antibody, Tocilizumab, For The Treatment Of Rheumatoid Arthritis

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Background: IL-6 is a proinflammatory cytokine and is known to play important roles in the pathogenesis of rheumatoid arthritis (RA). Several clinical studies have demonstrated that blockade of IL-6 signalling by a humanized anti-IL-6 receptor antibody is highly effective in the treatment of patients with active RA who are inadequately responsive to traditional anti-rheumatic drugs. IL-6 has a variety of biological activities that match many of the symptoms observed in RA patients. The precise mechanisms of action of tocilizumab are still not fully understood.

Methods: We reviewed possible mechanisms of action of tocilizumab, based on recently published papers.

Results: The data from Phase III clinical studies confirmed that tocilizumab can improve symptoms (including the number of swollen joints, the number of tender joints, fever, fatigue, anaemia and anorexia) in moderate to severe active RA. It has also been reported that tocilizumab prevented the radiographic progression of joint destruction. Tocilizumab has generally been well tolerated. Interestingly and importantly, serum IL-6 levels gradually decreased during long-term treatment, even though tocilizumab does not directly inhibit the synthesis of IL-6. This might be explained by the finding, in an animal model, that blockade of IL-6 signalling suppressed the induction of Th17 cells, which play a pathogenic role in the development of autoimmune diseases. In addition, we recently found that tocilizumab inhibited IL-6-induced RANK ligand expression on synovial cells, resulting in the inhibition of osteoclast formation. Tocilizumab also inhibited the gene expression of vascular endothelial growth factor (VEGF) which causes neovascularisation that increases the supply of oxygen and nutrition to growing synovial tissues.

Conclusion: Clinical studies have demonstrated that targeting the IL-6 signalling pathway with tocilizumab could be an attractive and innovative therapeutic option for RA. It is highlighted that high efficacy was achieved consistently in several studies, and this adds to the evidence for the deep involvement of IL-6 in the pathogenesis of RA.

In addition, blockade of IL-6 signalling inhibited the induction of Th17 cells and inhibited angiogenesis and bone destruction.

Challenges And Potential Solutions To Innovative Vaccine Development In Developing Countries

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Background: One of the barriers to meeting Millennium Development Goal Number 4 is access for large populations of developing countries to vaccines against most serious and common diseases. Vaccine development has been more readily available at affordable prices once more producers enter the market, but this will be a delay in access until these manufacturers can access the technology. Several solutions to enhance access have been proposed; none so far is optimal.

Methods: The factors relating to competition in the market and an affordable price were examined. Historical price data to countries were reviewed. Various explanations for failure of competition were also analyzed, including unequal access to financing, lack of optimal research and development capacity, barriers to technology transfer, issues in accessibility to intellectual property.

Results: Differences in vaccine scale up, know-how, GMP practices and regulatory oversight are decreasing between emerging suppliers and established multinationals. Developing country vaccine prices are at about the same levels for regulatory oversight are decreasing between emerging suppliers and established multinationals.

Conclusions: Based on these analyses, it was hypothesized that the interventions of the international community might be better directed to achieve vaccine access.

Enigmatic Eosinophil As Magic Bullet: Eosinophil-Induced Prognosis Improvement Of Solid Tumors Could Be Enabled By Their Vascular-Mediated Barrier Permeability Induction

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Background: Eosinophils are multifunctional cells, which contain and produce many biologically active substances. Generally, eosinophils are associated with parasitic infections and allergic disorders, while according to recent studies eosinophil infiltration is present also in target tissues of both physiological and pathological processes. With regard to solid tumors, the eosinophilic infiltration is associated with a better prognosis. In this review, the aim was to create a suitable hypothesis about this relationship

Methods: The relevant abstracts of PubMed publications are used in this review. Apart from abstracts reporting data about the relationship between mentioned tumors and eosinophils, to create the hypothesis are used abstract which describe eosinophil functions or abilities in diverse physiologic or path-physiologic situations.

Results: Reflecting on prognosis improvement in the case of solid tumors after eosinophilic infiltration of their capsules, it could be hypothesized that eosinophils are not tumoricidal per se; rather they can permeate such barriers through their vesicles’ content, whereas the tumidolymphokytinices such as interleukin 4 (IL-4) fulfill the tumor necrosis. This scenario can be supported by the fact that IL-4 originated from macrophages and lymphocytes helps to mess with many biologic functions that can be beneficial in vitro conditions in absence of eosinophils. In analogy with solid tumors, the requirement of eosinophil-mediated increasing permeability among diverse biologic barriers and tissues may explain the eosinophils’ introduction in capsules of cists, mucosal membrane of respiratory and gastroenteric systems, hematopoietic barrier, in embryos, as well as in bacterial and parasitic membranes.

Conclusions: In some situations such as solid tumors, rather than being multifunctional effectors per se, eosinophils, due to induction of target barrier permeabilization, may assure the inducted-created action, mediated by various kinds of leukocytes and their biologic effectors. Consequently, a better understanding of physiology and pathophysiology of this enigmatic cell will lead to new clinical strategies.
Selective Stimulation Of Human Natural Killer Cells Proliferation By Novel Fucosylated Acidic Glycan Drugs: Possible Therapeutic Use For Treating Cancer And Viral/Retroviral Infections

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Background: Definition of natural killer (NK) cells function is to kill cancer and viral/recovered infected cells. Selective stimulation of different subsets of NK cells proliferation is the prerequisite for specific and effective target killing. The nature of the molecules responsible for such stimulation was not completely established and consequently therapy using NK route could not be reliably achieved. Our aims were: 1) To find a new class of drug molecules which can induce selective proliferation of different subsets of NK cell populations in humans. 2) To widen and complement therapeutic methods for treating cancer and viral/recovered infections using NK cells as the "cellular magic bullet'.

Methods: 1) Fucosylated acidic glycans isolated by chromatography and electrophoresis from sponges were sequenced using NMR and MS. 2)  Ex vivo stimulation of human NK cell proliferation by these compounds was tested using peripheral blood mononuclear cells (PBMC) cultured in supplemented homologues serum for 1-3 weeks and measured by FAC S analyses using peripheral blood mononuclear cells (PBMC) cultured in supplemented homologues serum for 1-3 weeks and measured by FAC S analyses.

Results: 1) 33 novel fucosylated acidic glycan compounds were obtained. 2) Treatment of PBMC cultures with these compounds resulted in selective stimulation of CD4+ T lymphocytes, CD8+ T cells and NK cells, respectively. 3) Ex vivo killing of human tumor cells and viral infected cells with human NK cells were examined microscopically.

Conclusion: The obtained human NK cells showed massive and continuous killing of target human tumor or viral infected cells during five experimental periods.

Results: 1) Fucosylated acidic glycans isolate d by chromatography and electrophoresis from sponges were sequenced using NMR and MS. 4) Ex vivo stimulation of human NK cell proliferation by these compounds was tested using peripheral blood mononuclear cells (PBMC) cultured in supplemented homologues serum for 1-3 weeks and measured by FAC S analyses using antibodies for cell identification: CD3, TCR αβ, CD4, CD8, - T cells; CD16, CD56 - NK cells; CD107a - B cells; CD14 - monocytes. 3) Ex vivo killing of human tumor cells and viral infected cells with human NK cells were examined microscopically.

Results: 1) 33 novel fucosylated acidic glycan compounds were obtained. 2) Treatment of PBMC cultures with these compounds resulted in selective stimulation of CD4+ T lymphocytes, CD8+ T cells and NK cells, respectively. 3) Ex vivo killing of human tumor cells and viral infected cells with human NK cells were examined microscopically.

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Chemistry Of Nitrogen And Sulphur Based Compounds: An Approach Towards The Discovery Of Magic Bullets

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Background: The synthesis, physico-chemical investigation and biological studies of metal complexes of thiadimylene ligands are described which act as the "cellular magic bullet" for drug discovery. The main findings were: 1) The synthesized platinum (IV) and palladium (II) complexes of the thiadiimines and characterized by elemental analysis, IR, mass, electronic and H NMR spectroscopic studies. 2) To screen therapeutic compounds for antitumor and in vitro antibacterial activities. In vitro antitumor and in vitro antibacterial studies were performed against tumor and bacterial strains.

Results: The following scheme involves for the synthesis of thiadimylene ligand and their metal complexes

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\text{Ruthenium Complexes In Cancer Therapy: In Vitro And In Vivo Studies}
\]

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Background: Ruthenium-protein interaction leading to modulate the enzymes of cancer associated metabolic events is an evolving concept for anticancer, anti-infective and other therapeutic applications. Lactate dehydrogenase (LDH: EC 1.1.1.27) is found to be the critical enzyme implicated in maintaining the tumor growth via executing Warburg effect (increased glycolysis, and accumulation of pyruvate). Studies on the role of inhibition of MA-LDH in tumor development is quite evident (Koukourakis et al., 2003; Fantin et al., 2006), barring 'Warburg effect' in cancerous cells. Although implication of M4-LDH in tumor growth via executing cancer associated metabolic events is an evolving concept for anticancer mechanisms of the obtained findings.

Perfluorocarbons(PFC) As Universal Remedy Of Free Radical Homeostasis At The Wide Spectrum Of Various Metabolic Disorders

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Background: Routine biomedical applications for PFC and their emulsions include their use as a gas carriers, due to their ability transport and upload O2 and CO2. It's ability to dissolve great volumes of non polar gases (O2 and CO2) really dramatic. Oxygen solubility in PFC liquids is about 20-25 times greater than in either water or blood plasma under the same conditions. The PFCs are used in medicine as protective gases, because they transport better than normal gas mixtures by applying high pressure. In addition, their use is the most promising for the correction of metabolic disorders which caused or accompanied by free radical(FR) imbalance. In the work were discussed main mechanisms of the obtained findings.

Affiliates' disclosure statement: This abstract contains novel and unpublished data.

Perfluorocarbons(PFC) As Universal Remedy Of Free Radical Homeostasis At The Wide Spectrum Of Various Metabolic Disorders
EHRLICH II –2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the
Nobel Prize Award to Paul Ehrlich
Nürnberg, October 3-5, 2008
Aptamers As Magic Bullets And Delivery Vehicles In Disease Imaging And
Toll-Like Receptor (TLR) Agonists And The Induction Of The Innate Immune
Therapy
Response

MISSAILIDIS S

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1
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Background: The term of ‘magic bullet’, coined so long ago, would be most
appropriate for these novel targeting modalities termed aptamers. Aptamers are
nucleic acid in nature, and although they are larger than traditional chemotherapy
agents (~10kDa), they are smaller than antibodies and other protein-based
biologics. Furthermore, they have high affinity and specificity for their target,
significantly higher than that of small molecules or peptide and even antibody
therapeutics.
Method: Aptamers are usually selected by the SELEX methodology and we have
used anything between 1 and 10 round of selection and amplification to isolate
high affinity and specificity ligands. These are subsequently modified to ensure
resistance to nuclease degradation and their target affinity is assessed by SPR,
spectroscopic and immunological and immunohistochemical techniques, whilst
the pharmacokinetic and tumour penetration properties have been assessed in
experimental model systems.
Results: Aptamers are ideal magic bullets that can travel throughout the body
with minimal immunogenic or toxic effects, without entering cells that are not
carrying the aptamers’ cell-surface receptor target, bind specifically and inhibit the
action of their target. This has led to the success of aptamers in the market as
antiangiogenic agents with aptamers against VEGF for the treatment of macular
degeneration, and as inhibitors of nucleolin in clinical trials against cancer and our
own aptamers in preclinical studies with excellent tumour penetration properties.
Furthermore, where the cell-surface receptor is not part of a vital pathway and its
blockade does not lead to cell kill, aptamers have sufficient mass and relevant
pharmacokinetic properties to act as carriers of toxins, chemotherapy agents or
radionuclides to exert their therapeutic effects. Finally, this therapeutic action of
aptamers is complemented with their potential as diagnostic and imaging agents,
when their carrier allows distant recognition, such as gamma emitters, contrast
agents, NIR emitters or fluorescent agents. An overview of the aptamers’
characteristics and applications in disease diagnosis and therapy, examplified
with novel results from own research and that of other groups will be presented.

Background: TLRs are pattern recognition sensors that induce cellular immune
responses to the systemic presence of a variety of microbes. TLR agonists are in
active development as pharmaceuticals for a variety of indications. The most
advanced in clinical development are –CpG- (TLR9), LPS (TLR4), and dsRNA
(TLR3) agonists. Nine of the ten human TLRs utilize a common intracellular
signaling pathway. TLR3 employs a unique pathway that may be responsible in
part for its relative lack of toxic inflammatory responses. TLR3 ligands have been
demonstrated to have broad anti-viral, immunomodulatory, and anti-proliferative
responses in a wide spectrum of pre-clinical studies. The parent TLR3 agonist,
Poly I:Poly C, is not limited to TLR3 activation and is associated with a variety of
toxic responses in humans. The analog, Poly I:Poly C12U, is active only as a
ligand for TLR3 and is not associated with dose limiting toxicities which facilitates
clinical utilization for a variety of indications.
Methods: A variety of studies have demonstrated the utility of Poly I:Poly C12U as
an inducer of innate immune responses in the prophylaxis and treatment of
human diseases. These include:1) Phase 2 and 3 clinical trials for chronic fatigue
syndrome (CFS), 2) utilization as an immune enhancer for avian HPIV vaccines,
and 3) treatment of human renal cell carcinoma.
Results: 1) Double-blind, placebo-controlled, Phase 2 and 3 clinical trials with
Poly I:Poly C12U have demonstrated efficacy in primary end-points and a variety
of secondary end-points in well defined CFS. These include exercise tolerance,
decrease in drug usage for symptoms of CFS, and a decrease in physician and
patient assessments of disease symptoms. Poly I:Poly C12U was well tolerated; 2)
The intranasal administration of Poly I:Poly C12U with a Japanese seasonal
influenza vaccine in mice provided protection against challenge from the 3 viruses
represented in the vaccine as well as challenge with avian H5N1 HPIVs; 3) An
open-label study in human renal cell carcinoma (Elson Risk Group =3) was
compared to historical controls stratified to Risk Groups 1-5 over a 2 year
observation period. Poly I:Poly C12U provided significantly improved survival
compared to Risk Groups 2- 5.
Conclusions: Poly I:Poly C12U is a well-tolerated and active TLR3 agonist under
current clinical development for a variety of indications.
Authors’ disclosure statement: Studies on Poly I:Poly C12U have been funded
by Hemispherx Biopharma, by contract through NIH (NIAID/ NCI), or by the NIID
(Japan). WMM is an independent member of the BOD of Hemispherx Biopharma.

Helicobacter Cysteine-Rich Proteins A And -C, Two Novel Signalling
Molecules Form The Family Of Sel1-Like Repeat Proteins Involved In The
Modulation Of Pathogen/Host Interactions

Cadmium Stress Associated Protein Mediated Resistance to Fusarium
Infection in Wheat

MITTL P
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The spiral-shaped gram-negative ε-proteobacterium H. pylori settles in the gastric
lumen of primates and higher vertebrates and shows an exceptional adaptation to
its ecological niche, resulting in long-lasting infections of the gastric mucosa. The
persistence of H. pylori infections is most likely a consequence of its ability to
modulate the innate immune system of the host. To identify novel virulence
factors we initiated an inverse genetics approach, focusing on a family of secreted
proteins that are specific for å-proteobacteria. Due to the high content of disulfide
bridges this family was designated Helicobacter cysteine-rich proteins (Hcp). The
crystal structures of HcpB and -C served as the prototype structures for the large
family of Sel1-like repeat proteins that participate as protein/protein-interaction
modules in signaling pathways of bacteria and eukaryotes but not in archea.
Expression of Hcps under in vivo conditions was confirmed by the detection of
elevated anti-Hcp IgG titres in H. pylori positive patients. Since Hcp knock-out
experiments suggested that Hcps might be important for the interaction between
the bacterium and its host, we investigated the effects of recombinant Hcps on
mammalian leukocytes. HcpA elicits the release of cytokines from naive mouse
splenocytes and also affects the morphology and adherence of monocytes in
vitro.
The sequence analysis of Hcp homologues from a large collection of H. pylori
strains uncovered amino acid positions that seem to be important for the
functionality of Hcps and for the adaptation to different host populations.
Thus, Hcp serve as bacterial signaling molecule that modulate important
properties of mammalian immune cells. To gain deeper insights into the
adaptation of H. pylori to different host populations we are currently working
towards the analysis of the Hcp signal transduction mechanisms. Recent progress
in the identification and validation of eukaryotic Hcp receptor molecules will be
discussed.

MITTRA B1 , GHOSH P2, HENRY SL3, PATTNAIK SS4 , DAS TK5 , GHOSH S6,
BABU CR7 AND MOHANTY P8
1,4
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University,India52001, India;8Regional Plant Resource
Center,Bhubaneswar,India.
Background: Plants do not have a defined immune system directly comparable to that of animals. However, plants
respond to pathogen attack by a variety of biochemical means known as defense responses. Particularly, during fungal
infection, plants synthesize low molecular mass inhibitory compounds such as, phytoalexins and accumulate pathogenrelated (PR) proteins. In many plants, enhanced disease resistance is accompanied by the activation of genes encoding
pathogen-related (PR) proteins [30]. Recently, the defense-role of sugarcane glycoproteins against the smut disease in
sugarcane caused by Ustilago scitaninea was demonstrated. Similarly, there has been increasing evidence for direct
influences of strobilurins, a fungicide, on plant defense physiology, which suggest that in addition to their fungicidal activity,
strobilurin also enhances the capability of plants to ward off viral and bacterial pathogens. The anti fungal activity of a
strobilurin compound, F-500 (Pyraclostrobin) has been tested in tobacco plants which, enhanced plant resistance against
tobacco mosaic virus (TMV) and bacterial pathogen, Pseudomonas syringae pv tabaci . Some chemicals and metal ions
are anti fungal in that the treatment of seeds prevents or arrests fungal infection to plants. Cadmium salts are usually used
as antifungal . However, cadmium is also phytotoxic, athigh concentration it retards plant growth and development .
Exposure of plants to heavy metals like Cd2+ induces production of phytochelatin and some stress associated
proteins,SAP. However, no report has been found on Cd2+ pre-exposure imparting resistance to fungal specification .In
this communication, we report, for the first time, on a novel mode of antifungal activity of CdCl2 in that a pre-exposure of
wheat seedlings to a mild dose of CdCl2 imparts immunity to plants against Fusarium infection.Aims:1) Structural
characterization of cadmium associated stress protein(CSAP) induced in wheat with the treatment of a low dose CdCl2
.2)To assess the possible direct and/or indirect role of glutathione(GSH) against fungal infection in wheat apart from its
heavy metal detoxification activity.
Method: Surface sterilized wheat (Triticum aestivum L.) seeds(100), treated with 0.01 % HgCl2 (m/v) for 10 min and
washed thrice in sterile distilled water, were treated with50 µM CdCl2 for 48 h at room temperature (28 ± 2 °C).The seeds
were allowed to germinate on filter paper saturated with sterile distilled water. An equal number of seeds without Cd2+
treatment served as control. The Cd content of the control and the treated seedlings were estimated by atomic absorption
spectrometry. Seven days old untreated (control) and CdCl2 pre-exposed seedlings with a well developed root system
were grown in test tubes containing liquid MS (1/10 strength) medium supplemented with 4-day-old freshly cultured F.
oxysporum (1x106) inoculums. The mild dose Cd2+ pre-exposed seedlings maintained in tubes containing F. oxysporum
with MS basal medium were considered as test plants while the only Cd2+-exposed seedlings served as comparative
control. Seedlings without Cd2+ pre-exposure (control) and seedlings grown in only F. oxysporum inoculums with MS basal
medium were also maintained for comparison .A total 48 number of seedlings were used per each set of experiment and
repeated for three times. All test and control plants were incubated at 26 °C ± 2 °C .Two seedlings per tubes were
considered for better observation. The tolerance of the plant assessed was based on the survival and growth of the
seedlings under stressed conditions. Growth and survival data were recorded for a period of another seven days.
Fourteen days old germinated seedlings (500 mg) were removed from the seeds and crushed with 10mM sodium
phosphate buffer (pH 7.55). Samples were then centrifuged at10000 rpm (2,500 g) using a centrifuge for 10 min. (Remi).
The supernatant containing protein was collected and pellet was washed, re-centrifuged and then discarded. Protein was
estimated according to Lowry et al. Protein samples were dialyzed against the same phosphate buffer with two changes for
24h each and finally concentrated by using polyethylene-glycol (PEG-60).
Extracted protein was characterized on a 10% SDS-PAGE and the molecular weight of the particular protein band of
control (without treatment), Fusarium treated, Cd2+ (50µM)-exposed and co-stressed (50µM Cd2+ pre-exposed and then
infected with F.oxysporum) seedling was determined from their Rf values. The particular protein band of interest, which
was expressed dominantly due to stress induction, was cut out from the particular gels and the protein was electro-eluted in
a Bio-rad (Electro-eluter) apparatus, using the electro-elution buffer (Tris 25mM, glycine 192mM and SDS 0.1%, pH 8.3) at
a constant current of 8-10mA/glass tube for 5h. The eluted protein was collected, and concentration was estimated and
again ran on a 10% SDS-PAGE to establish its homogeneity. Further in-gel triptic digestion and liquid chromatography
mass spectrometry (LC-MS/MS) analysis was done. The purified protein was dissolved in water at a concentration of 80 µg
cm-3. The N-terminal sequence ofCSAP was obtained by automated Edman degradation,followed by HPLC and UV
detection (Edman and Begg1967), using a PPSQ-21A protein sequencer (Shimadzu,Kyoto, Japan). Searches for sequence
similarity were performed using Blast P databases.
[Regrettably not enough space on this page.]

All abstracts are listed in alphabetical order of the presenting author.
Abstracts

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Mechanism Of Oxidative DNA Damage And Apoptosis Induced By Doxorubicin Through Generation Of Reactive Oxygen Species

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Background: The anticancer mechanism of doxorubicin (DOX), an anthracycline antibiotic, is believed to involve DNA damage and apoptosis through topoisomerase II inhibition and reactive oxygen species (ROS). However, the precise mechanism of DNA damage and apoptosis induced by DOX remains to be clarified. We investigated the mechanism of DNA damage and apoptosis induced by DOX.

Methods: We used human leukemia cell line HL-60 and its H2O2-resistant HP100 cells. Apoptosis induced by DOX was detected by DNA ladder formation. Detection of peroxide and in cells treated with DOX was analyzed on a flow cytometer. Measurement of caspase-3 activity was used by DEVD-AFC, a caspase-3 synthetic substrate. Measurement of 8-oxodG, a marker of oxidative DNA damage, was used by HPLC-ECD. In addition, we analyzed DOX-induced DNA damage, using 32P-labeled DNA fragments.

Results: DOX-induced DNA ladder formation could be detected earlier in HL-60 cells than in HP100 cells, suggesting the involvement of H2O2-mediated pathways in apoptosis. Flow cytometry revealed that H2O2 formation preceded the increase in m and caspase-3 activation. Poly(ADP-ribose) polymerase (PARP) and NADPH oxidase inhibitors prevented DOX-induced DNA ladder formation in HL-60 cells. Moreover, DOX significantly induced formation of 8-oxodG, in HL-60 cells. HP100 cells. DOX-induced apoptosis was mainly initiated by oxidative DNA damage in comparison with the ability of other topoisomerase inhibitors to cause DNA cleavage and apoptosis. Moreover, DOX caused site-specific oxidative DNA damage in the presence of rotenone(II), which may contribute to apoptosis.

Conclusions: These results suggest that the critical apoptotic trigger of DOX is considered to be oxidative DNA damage by the DOX-induced direct H2O2 generation, although DOX-induced apoptosis may involve topoisomerase II inhibition. This oxidative DNA damage causes indirect H2O2 generation through PARP and NADPH oxidase activation, leading to the m increase and subsequent caspase-3 activation in DOX-induced apoptosis.

Antimicrobial Resistance In Major Pathogens Of Surgical Site Infection In Iran Hospitals

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Background: The 2006-07 Swaziland Demographic and Health Survey (SDHS) was a national-level sample survey which was implemented by the Central Statistical Office (CSO) at the request of the Ministry of Health and Social Welfare (MOHSH). The principal objective of the survey was to provide up-to-date information on prevalence of HIV among other things. The aim was to identify behaviours that protect or predispose the population to HIV infection, examine social, economic, and cultural determinants of HIV, and determine HIV prevalence among males, females and children age 2 years and older.

Methodology: Children age 2 to 14 years and older adults 50 years and above who were household members aged 15 years and older were interviewed. Children aged 15-17 were also asked to provide consent in addition to obtaining parental consent. The data was processed by office editing, coding of open-ended questions, data entry, double-entry verification, and resolving inconsistencies found by computer programmes developed for the SDHS. The SDHS data entry and editing programmes used CSiPro, a computer software package specifically designed for processing survey data such as that produced by DHS surveys.

Results: The results indicate that 26 % of adults are HIV infected; 31% of women and 26% of men are infected. Prevalence is higher in urban than rural areas, 37% and 29% respectively. Almost one in two women aged 25-29 is HIV-positive. Prevalence is highest among those who are divorced, separated, and widowed. Prevalence increases with number of lifetime partners and 17% of couples were discordant.

Conclusion: This information provide data to assist policymakers and programme implementers to monitor and evaluate existing programmes and to design new strategies for demographic, social and health policies in Swaziland. The survey also provides data to monitor the country's achievement towards the Millennium Development Goals.

Swaziland Demographic and Health Survey 2006–2007 with Special Focus on the Prevalence of HIV

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Objective: Surgical site infections are a significant problem which limits the potential benefits of surgical interventions. The impact on hospital costs and postoperative length of stay is considerable. The increased occurrence of antimicrobial-resistant microorganism is a major medical concern. We determine the resistance to antibiotics in major pathogens isolated from surgical site infection in our hospitals in Isfahan, Iran.

Methods: Antimicrobial susceptibility of bacterial isolated from surgical site infection in two university hospitals from 2005 to 2006 was monitored by Etest(R) test. Enterobacter and Pseudomonas aeruginosa were the most common infective microorganisms. A antimicrobial use committee. Surveillance of antimicrobial resistance should be an important intervention.

Conclusions: In Iran, like other countries antimicrobial resistance is a serious clinical problem among healthcare facilities. Each healthcare facility should have an antimicrobial use committee. Surveillance of antimicrobial resistance should be improved and antimicrobial restriction is an important intervention.
The Psychological Outcome Of Male Constitutional Delay Short Stature

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Background: Growth is said to be the defining feature of childhood, with genetic and environmental factors influencing the rate of growth and the final stature. Studies have demonstrated that idiopathic short stature, familial short stature and constitutional delay of growth male children often respond to growth treatment: Mental health outcome studies of male short stature have varied but these studies have been difficult to compare due to differences in cohort age groups and differences in assessment tools. However past studies have investigated psychological outcome as being dependent on height (short versus tall).

Methods: A novel approach was devised to measure how adult male final height was dependent upon the mental health status for those treated (Rx+; n=27) and untreated (Rx−; n=21) with growth promoting products as children defined as having constitutional delay. The respondents were assessed by the BCL-90-R psychological distress and psychopathology instrument and allocated into either a clinical, distressed group, or a non-clinical, non-distressed group. The Rx+ respondents had received as children (1) oral oxandrolone or (2) intramuscular injections of testosterone esters; other oral preparations may have been used but growth hormone was not used.

Results: (1) The main finding was that there was a significant relationship between psychological distress and height outcome for the group (Rx+ Rx−) in psychological distress and the group (Rx Rx−) not in psychological distress. Chi-Square p<0.03. (2) The average gain in height from treatment in the non-psychologically distressed group was 1.8 cm and the for psychologically distressed group 1.4 cm.

Conclusion(s): (1) Those not in psychological distress were height advanced. Had the psychological state not been controlled then the outcome findings would have been decidedly confounded. (3) The height improvement for the treated cohort can be compared with similar modest increases using recombinant growth hormone for growth promotion in other studies. (4) When male children present for investigation and possible treatment for growth promotion they should be assessed for their mental health status as this may well be a predictive factor on adult height outcome which further longitudinal studies may resolve.

Magnetic Nanoparticles For Controlled Delivery Of Methotrexate

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Background: Cancer is a major cause for morbidity and mortality in industrialized countries. For patients with advanced disease, chemotherapy based on methotrexate is a mainstay of treatment. We have developed a new water-dispersible oleic acid (OA)-Pluronic-coated iron oxide magnetic nanoparticle formulation that can be loaded easily with high doses of water insoluble anticancer agents.

Methods: Nanoparticles of iron oxide were synthesized by mixing Aqueous solution of 0.1 M FeCl3 (30 mL) and 0.1 M FeCl2 (15 mL) and dropwise adding of 3 mL of 5 M ammonia solution over 1 min under continuous stirring on a magnetic stirrer. The magnetic nanoparticles were coated with Pluronic, which is an amphiphilic block copolymer of polyethylene glycol (PEG) and polypropylene glycol (PPG). To study the effect of OA, formulations with different weight ratios of OA to iron oxide nanoparticles were prepared. For this purpose, OA was added from 60-240 mg corresponding to same weight of iron oxide nanoparticles. After the coating of nanoparticle with pluronic, methotrexate was loaded to particles.

Results: The loading efficiency was more than 90 % in optimized condition. Neither the formulation components nor the drug loading affected the magnetic properties of the core iron oxide nanoparticles. The release pattern was uniform in water for 8 hrs under in vitro conditions.

Conclusion: This system could be used to deliver anticancer drug methotrexate to the tumor site by using an appropriate external magnetic field. Based on the release pattern it would be practical to target the drug in higher concentration in tumor site than the other sites of the body.

Trial Of Lamivudine In Hepatitis B Surface Antigen Carriers With Persistence Hepatitis B Core IgM Antibody

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Background: The persistence of hepatitis B core IgM antibody in hepatitis B surface antigen carrier is a risk factor with hidden danger and forecast existence of liver damage. A trial of lamivudine in such subset of carriers was carried on for the first time in this study.

Methods: A total of 62 hepatitis B surface antigen with hepatitis e antibody individuals (age range, 25-45 years) with persistence hepatitis B core IgM antibody were randomized to receive either 100 mg lamivudine (32/62) or placebo (30/62) daily for 6 months.

Results: Among lamivudine group, hepatitis B core IgM antibody seroclearance achievement rate was 81.3% and HBsAg seroconversion rate was 9.4 % compared to 6.3% and 3.3% in placebo group respectively. A number of adverse clinical events were observed, but were of mild nature and tolerable by the participants who completed the study.

Conclusion: Trial of lamivudine in this subset of hepatitis B surface antigen carriers proven to be safe and efficacious. More studies are needed prior to recommending the drug for routine use on selected HBV carriers.
Plant Alkaloids Bioactive Molecules On A Wide Range Of Organisms

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Along human history, different civilizations have traditionally used many plant species both as herbal medicines and as stimulants. The molecular activities that are responsible for these effects typically arise from metabolites present in plant tissues. In many of these herbal remedies alkaloids are present as bioactive components.

In recent years, it has become apparent that there are novel signaling molecules, which might play important roles in the regulation of morphogenetic and adaptive processes. Recent work with A. thaliana has provided evidence of the potent biological activities of some alkaloids, such as aflatoxin. Alkaloid and the interaction with cannabinoid receptors, which are coupled to signal. Hence, there is a possibility that cannabinoid signaling represents an evolutionarily conserved pathway that modulates cellular and physiological processes in eukaryotes. In plants the formation of NAEs, structurally related to alkaloids and to anandamide, were initially associated with germination and responses to pathogen attack. This information in the lab showed that alkaloids in lower concentrations, but not NAEs, greatly stimulate root and aerial parts development, however both groups of compounds in higher concentrations inhibit plants development. Two aflamin-derivative alcohols and two indole alkaloids were found to be even more active than aflatoxin in stimulating cell responses. Unsaturated alkaloids have been shown to be fungitoxic and bacteriostatic. Additionally, in the Arabidopsis seedlings incubated in vitro, an increased expression of defense response genes occurred in parallel with accumulation of salicylic acid and jasmonic acid.

Cell Cycle Kinases As Molecular Targets In Anticancer Therapy

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Methods: Small molecule kinase inhibitors were identified by screening a proprietary kinase targeted library of approximately 50,000 compounds using recombinant kinases in a biochemical screening format. After reconfirmation, hits were screened for cellular activity and mechanism of action in vitro and in vivo. Efficacy and PK/PD modeling of lead compounds were assessed in different tumor animal models. Toxicity was assessed in primary and secondary non-rodent species.

Results: Upon screening our chemical library for Aurora, Polo and CDC-7 kinases we have identified potent and selective hits. These hits were optimized and SARs were developed. Upon lead optimization, small molecules were identified for all targets and were found to be highly efficient in vivo and are well tolerated. The most advanced compound, PHA-739558, was the first Aurora kinase inhibitor to enter clinical trials and is currently in phase II. An additional clinical opportunity is based on its cross-reactivity with wild-type and T315I mutant BCR-ABL, which will be shown and discussed.

Conclusions: Small molecules inhibiting different cell cycle kinases have potent anti-tumor activity, however the cellular responses and mechanisms of action are distinct for inhibitors of Aurora, Polo and CDC-7 kinases. This difference might be important for treatment of different indications or subsets of patients. It is expected that small molecules inhibiting these targets will add to the future armory of available anti-cancer agents.

Authors’ disclosure statement
All authors are full-time employees of Nerviano Medical Sciences Srl.
Expression Of MAGE-A12 In Oral Squamous Cell Carcinoma

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Background: Melanoma-associated-A antigens (MAGE-A) are silent in normal tissues except tests. However, they are activated in a variety of different tumors. Thus, their expression is highly specific to cancer cells. Reverse transcription-

Exon 5 of rabbit SERCA 1a at 3.3 Å resolution shows that the long flexible hydrocarbon between the M3, M5, and M7 transmembrane segments of the Ca2+-ATPase has resulted in the elucidation of a vast number of the structures of this protein at atomic resolution, corresponding to its various phosphorylated / non-phosphorylated and inhibitor bound states. Thus the specific high-affinity binding site of the sesquiterpene lactone thapsigargin (TG), isolated from the mediterranean plant Thapsia garganica, to the E2 conformations of SERCA 1a has been found in a preformed notch, located at the lipid-protein interface between the M3, M5, and M7 transmembrane segments of the Ca2+-ATPase (Olesen et al. 2004, 2007). Although the inhibition of SERCA pumps in both normal and cancer cells precludes the use of TG as an antineoplastic agent, this inhibitor has been used successfully for the detection of MAGE genes in tissue samples. Aim: To analyze the expression rate of MAGE-A12 in oral squamous cell carcinoma (OSCC) using a high sensitive RT-nPCR.

Methods: Total of 57 tissue samples obtained from patients with OSCC and 20 normal oral mucosal (NOM) probes of otherwise healthy volunteers were included to this study.

Results: No expression of MAGE-A12 was observed in the non-neoplastic NOM tissues. MAGE-A12 was expressed in 49.1% of the investigated tumor samples. The correlation between malignant lesion and MAGE-A12 detection was significant (p < 0.001).

Conclusions: 1) Results of this study may indicate MAGE-A12 as a useful additional diagnostic marker especially for the early detection of OSCC distinguishing neoplastic transformation and detection of occult and/or rare disseminated cancer cells. 2) MAGE-A12 expression in OSCC may also determine a new immunotherapeutic target and might be warranted to develop vaccine for OSCC.

Development of SERCA Inhibitors Targeted towards Prostate Cancer Cells

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The ability to produce well ordered crystals of sarcoplasmic reticulum (SERCA) Ca2+-ATPase has resulted in the elucidation of a vast number of the structures of this protein at atomic resolution, corresponding to its various phosphorylated / non-phosphorylated and inhibitor bound states. Thus the specific high-affinity binding site of the sesquiterpene lactone thapsigargin (TG), isolated from the mediterranean plant Thapsia garganica, to the E2 conformations of SERCA 1a has been found in a preformed notch, located at the lipid-protein interface between the M3, M5, and M7 transmembrane segments of the Ca2+-ATPase (Olesen et al. 2004, 2007). Although the inhibition of SERCA pumps in both normal and cancer cells precludes the use of TG as an antineoplastic agent, this inhibitor has been used successfully for the detection of MAGE genes in tissue samples. Aim: To analyze the expression rate of MAGE-A12 in oral squamous cell carcinoma (OSCC) using a high sensitive RT-nPCR.

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Trans-Cinnamaldehyde From Cinnamomum Zeylanicum Bark Essential Oil Reduces The Clindamycin Resistance Of Closstridium Difficile In Vitro

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Background: Therapy with antimicrobial drugs, such as clindamycin, that perturb the intestinal flora but fail to inhibit growth of other microorganisms can permit the proliferation of Closstridium difficile and the elaboration of exotoxin. Therefore, there has been increasing interest in the use of inhibitors of antibiotic resistance for use in combination therapy. The essential oil of Cinnamomum zeylanicum bark enhanced the bactericidal activity of clindamycin and decreased the minimum inhibitory concentration of clindamycin required for a toxicogenic strain of Closstridium difficile.

Methods: Thin-layer chromatography (TLC) analysis of the essential oil separated a fraction (Rf, 0.54) that was the most effective at enhancing the clindamycin antimicrobial activity. Using gas liquid chromatography and known standards, the active fraction was identified as trans-cinnamaldehyde (3-phenyl-2-Propenal). Combinations of clindamycin and trans-cinnamaldehyde were tested to determine the fractional inhibitory concentration (FIC) index by conventional checkerboard titration. The FIC index for Closstridium difficile was found to be 0.312, which confirmed the synergistic actions of clindamycin and trans-cinnamaldehyde.

Results: The presence of 20 µg/mL of trans-cinnamaldehyde decreased the MIC of clindamycin for Closstridium difficile.

Conclusion: low concentrations of trans-cinnamaldehyde elevate the antimicrobial action of clindamycin, suggesting a possible clinical benefit for utilizing these natural products for combination therapy against Closstridium difficile.

Inhibition Of Drug Resistance Of Bacteria And Cancer Cells

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Background: Chemotherapy started with Ehrlich’s “Magic Bullets” and large numbers of antibiotics were developed on the basis of his ideas. Resistance to antibacterial and anticancer drugs appeared soon after the introduction of antibiotics and chemotherapeutics, resulting in non-treatable infections and malignancies. This paper reports on the reversal of the multidrug resistance (MDR) of bacteria and tumor cells.

Methods: Plasmid replication, partition and conjugal transfer were inhibited simultaneously in various bacterial species carrying plasmids encoding antibiotic resistance in the presence of phenothiazines and disiloxanes in vitro. The inhibition of the ABC transporter of the cancer cells was measured in the presence of phenothiazines and patented disiloxanes by rhodamine accumulation in flow cytometry in human MDR1 gene-transfected mouse lymphoma, human breast and colon cancer cell lines.

Results: Antiplasmid effects of substituted phenothiazines and structurally related compounds were demonstrated on Gram- positive and negative bacteria. Elimination of the antibiotic resistance of R plasmids of E. coli and other bacterial species was induced by phenothiazines. The effects of antiplasmid compounds are based upon intercalation into the superhelical form of plasmid DNA and the simultaneous inhibition of conjugal plasmid transfer. The phenothiazine promethazine synergized the antibacterial effect of gentamycin in chronic recurrent pyelonephritis in vivo. On the basis of structure-activity correlations, anthrilot-derivatives with antiplasmid activity were synthesized.

MDR is the main reason for the failure of cancer chemotherapy. The MDR1 activity of human MDR1 gene-transfected mouse lymphoma, human breast and colon cancer cells was inhibited in the presence of resistance modifier phenothiazines and disiloxanes. The disiloxane modifiers synergized the antiproliferative effect of doxorubicin and taxol in vitro. In addition the disiloxane reduced the growth rate of cancer cells co-cultivated with normal cells (unpublished results).

Conclusions: The results of model experiments on the synergistic interactions between antibiotics, chemotherapeutics and resistance modifiers on bacteria and cancer cells can be exploited in rational drug design for combination chemotherapy.
First-Line Treatment With Rituximab Combined With Intravenous Or Oral Fludarabine For Patients With Extranodal Mucosa Associated Lymphoid Tissue (MALT) Lymphoma

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Methods: This study enrolled 22 adult patients with extranodal MALT lymphoma, who had not received previous chemotherapy and who require systemic treatment. Patients received Rituximab 375 mg/m² intravenously (IV) on day 1 and Fludarabine 25 mg/m² (IV) on days 1-5 (days 1-3 when > 60 years), every 4 weeks; after the first cycle, oral Fludarabine was allowed to be given orally at 40 mg/m² with the same schedule. An evaluation was done after three cycles and patients in complete remission (CR) received an additional cycle and those achieving partial remission (PR) for a total of 6 cycles.

Results: Of 18 included patients, 10 (55.5%) achieved CR, 2 (11.1%) achieved partial remission (PR), and 6 (33.3%) achieved stable disease (SD). The overall response rate was 77.8% (CI95%: 70-90%). At 12 months, 9 patients (50%) remained in CR and 2 in PR. All patients received 2 cycles of treatment, and patients with complete remission (CR) received an additional cycle and those achieving partial remission (PR) received a total of 6 cycles.

Conclusions: From the study, it was concluded that bulky substitutions in the 2nd position of DBO increased the activity than parent compound. In DBS, 2 substituted compounds have significant activity against the microbial strains than substituted in 1st position. Except naphthalene, other compounds have negligible activity against E. coli and maximum activity with B. subtilis. Chloro, dichloro and hydroxyl derivatives of TZ have significant activity at 64 mg/ml against C. albicans.

In silico PK prediction shows that all the compounds obeyed Lipinski rule of 5 and have free of toxicity and metabolically stable.

Conclusion: From the study, it was concluded that bulky substitutions in the 2nd position of DBO increase the activity than parent compound. In DBS, 2 substituted compounds have significant activity against the microbial strains than substituted in 1st position. Except naphthalene, other compounds have negligible activity against E. coli and maximum activity with B. subtilis. Chloro, dichloro and hydroxyl derivatives of TZ have significant activity at 64 mg/ml against C. albicans.
Background: How can an efficient low-to-toxic adjuvant nanoparticle concept rest on the same concept as a cancer cell killing drug? The 40 nm ISCOM nanoparticles are based on acyl-sapogenin (ASAP) and deacyl-sapogenin (DSAP) from Quillaja saponia (QS). They act as their antigen by guiding cells of lymphoid origin to activation measured by down regulation of CD14 on immature dendritic cells (DCs) and expression of CD83 to differentiation measured by production of proinflammatory cytokines and expression of communication molecules e.g. CDB. That is a normal pathway leading to the programmed cell death i.e. apoptosis virtually without any side effects. More than 450 publications have demonstrated that ISCOM formulations are potent adjuvants with strong immunomodulating capacity. ISCOMs are now in human phase 3 studies. To note the free i.e. non-particulate QS and the ASAP fraction are highly cell lytic due to interaction between the saponin and the cholesterol in the cell membrane. By saturating the acyl-sapogenin with cholesterol non-toxic particles can be formed like ISCOMs that are well tolerated by man and animal.

Methods: The present immune adjuvant and cancer cell killing concept is developed from the ISCOM technology used as adjuvant formula. Two formulations i.e. one containing ASAP and the other containing DSAP were used. A blocking technique was applied to analyse interaction of QS nano-particles with cancer cells revealing two receptor specific effects. Various methods were used to demonstrate virus-like and to apoptotic cell death as well as the exit of the cancer cells from the cell cycle. Various cancer cells including leukemic cells and solid tumour cells were exposed to the KGI particles and the cell survival was measured by the Alamar Blue and also other methods.

Results: The nanoparticle KGI and BBE mediate their effects by communicating with the target cells by a primary attachment receptor common to ASAP and DSAP having a functional modulating capacity and a second receptor unique for ASAP most likely located on the acyl-chain. ASAP in the KGI has an additional strong modulating device for apoptosis different from the device of the communication between KGI and BBE. The immune modulation and cancer cell killing effects of ASAP (KGI) and DSAP (BBE) particles differ resulting in synergistic and antigenic cancer killing effects and also they potentiate the effect of standard anticancer drugs measured in vitro. The KGI and BBE particle groups have shown cancer cell killing effect on cells from about 10 different lines including cells from solid tumours.

Conclusion: QS particles can be used as vaccine adjuvant and as anti-cancer drug following the same iso-structural function i.e. induction of activation, differentiation and eventually apoptosis. 1) The dual KGI - BBE system is a new low-to-non-toxic concept applicable for immune adjuvants, which has been validated as well as the concept being extended to a high therapeutic index, which is a new discovery. 2) The dual particle system has been shown in vitro to kill a variety of cancer cells including cells from solid tumours. 3) The particles can also act synergistically with other standard anti-cancer drugs. In short, the dual ASAP and DSAP particle concept is an efficient, low-toxic, highly bioavailability vaccine adjuvant and cancer killing system based on initial normal cell differentiation. The stand alone and synergistic effects between the components of the system, provides great promise for strong developments in two medical fields.

Pharmacokinetics (PK)/Pharmacodynamics (PD) Of Inflliximab In Treatment For Patients With Rheumatoid Arthritis: Characterization Of Inflliximab-Resistant Cases And PK-Based Modified Therapy

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Background: Inflliximab, a chimeric anti-tumor necrosis factor α (TNFα) monoclonal antibody, has provided a significant impact on management of rheumatoid arthritis (RA) however, a subset of RA patients show poor therapeutic responses. The purpose of this study is to understand the mechanism underlying such observations and to develop clinical PK/PD properties of infliximab and to present modified therapy for the patients with rheumatoid arthritis (RA). The main immunosuppressive mechanisms of infliximab are the induction of cell apoptosis and the inhibition of lymphocyte activation. However, the treatment outcomes of RA patients varied widely.

Methods: Twenty-one patients with active RA were scheduled to receive an intravenous infusion of infliximab (3 mg/kg or 200 mg) at weeks 0, 2, and 6, followed by maintenance therapy every 8 weeks. We examined a relationship between clinical responses and trough serum concentrations of infliximab at week 14. The in vitro cytotoxic effects of different concentrations of infliximab on cancer cell lines were examined at 24 h and assessed by the number of viable cells and cell death rate. The inter-individual and intra-individual PK data was examined using non-compartmental analysis.

Results: Fifteen cases achieved good or moderate responses in the European League Against Rheumatism criteria, and 3 cases resulted in nonresponders at week 14. The end of serum half-life (t1/2) during the first 2 weeks were 0.55 L/kg and 9.5 days, respectively. Among 14 weeks, most patients showed undetectable levels of trough serum concentrations of more than 1 µg/mL. Only 3 cases showed undetectable levels of trough serum concentration at week 14. By contrast, the PK profiles of all nonresponders except one showed rapid clearance during therapy. We also found that the t1/2 during the first 2 weeks is inversely correlated to the disease activity scores at the start of therapy. For patients with a rapid clearance of infliximab, the t1/2 was increased to 105 days, whereas the increased t1/2 of prednisone or methotrexate was a useful way to achieve sufficient clinical responses. The addition of tacrolimus was effective to improve the clinical outcomes of RA patients.

Conclusions: (1) Maintaining the trough serum concentrations of infliximab above therapeutic limit levels is beneficial for favorable clinical outcomes. (2) The rapid clearance appears to be the main course of unresponsiveness of infliximab. (3) PK data apparently offer guidance when an optimal treatment for infliximab-resistant RA patients is being considered.

Cytotoxic Diterpenes From Australian Flacourtiaceae As A Good Source Of Antineontic Agents

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Background: A new anti-cancer drug from an Australian Flacourtiaceae plant, which is a unique source of diterpenes, has been isolated from root of Casearia multinervosa. The plant has also been isolated from root of Casearia multinervosa. The plant has been used in traditional medicine for the treatment of cancer.

Methods: In this study, the in vitro cytotoxicity of the diterpenes isolated from the plant was evaluated using the MTT assay method. The IC50 values (µM) were determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: The described in-line processing, i.e. the conversion of an anti-cancer drug into a new anti-cancer drug, has shown very promising results. The new anti-cancer drug has shown high potency against various cancer cell lines, including breast, prostate, and lung cancer cell lines.

Conclusions: The diterpenes isolated from the plant have shown promising anti-cancer activity. Further studies are needed to determine the mechanism of action of these diterpenes in cancer cells.
Role Of Ultrasound Waves In Enhancing The Effect Of Antitumor Drug

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Background: Chemical activation of drugs by ultrasound for treatment of cancer is a new field recently termed "sonodynamic therapy". The combination of a drug and ultrasound has a killing effect on the cell. Despite this fact very little is known about structural changes of tumor cells after treatment with anticancer drug and ultrasound. It is now recognized that the contribution of sonochemistry in tumor cells is poorly investigated. Aims: 1) To evaluate the role of ultrasound in enhancing the effect of free radical damage during sonoevaporation. 2) To study the physical parameters, which enhance ultrasound effect on anticancer drug transportation through biological membrane. 3) To propose optimized dosages regions of application.

Methods: To evaluate the role of ultrasonic waves in enhancing the effect of 5-fluorouracil (5-FU) as anticancer drug, tumor growth, cell structure and temperature rises of ascites Ehrlich tumor implanted in mice were investigated at a frequency of 0.8 MHz. Different conditions of ultrasonic intensity (1, 2 and 3 W/cm²) and durations (1, 3 and 5 min) were studied. A total of 10 mg/kg body weight of 5-FU was i/v injected into the mice bearing Ehrlich tumors on days of 1, 3, 5, 8 and 10 of therapy. After 24 h of each injection of 5-FU, tumor sites were sonicated at room temperature of 22°C. Tumor volumes were monitored by using ultrasonic imaging system during treatment just before each 5-FU injection, and on the 15th day of treatment. Density and ultrasonic attenuation of excised tumor tissues were measured in vitro and used to estimate the temperature rises due to ultrasonic absorption. Results: Tumor volumes were monitored by using ultrasonic imaging system during treatment just before each 5-FU injection, and on the 15th day of treatment. Density and attenuation coefficients of excised tumor tissues were found to be dependent on the treatment regimen. The estimated rate of rise and equilibrium temperature, and the characteristic time to reach equilibrium are given for each group. Results obtained indicate that tumor growth decreases with increasing of ultrasonic intensity and sonication time. Tumor growth was delayed 4 to 6 days by combined treatment of 5-fluorouracil and ultrasound (US). Ultrastructural investigations of tumor cells showed several damages in cytoplasmic organelles and cytoplasmic vacuoles that increased with increasing ultrasonic intensity and sononation time. This damage appears as prominent crowded vacuoles among swollen ruptured organelles, chromatid fragments and severe increase in numbers of pyknotic and apoptotic cells.

Conclusions: The combination of 5-FU and ultrasound produced significantly greater antitumor activity than ultrasound or 5-FU alone.

Conclusions: The combination of 5-FU and ultrasound produced significantly greater antitumor activity than ultrasound or 5-FU alone.

Investigations Into Sulfur Nanoparticles As Drug Carriers

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Background: Inorganic nanoparticles are used in the field of in vivo diagnostic nuclear medicine practice. E.g. radiolabeled sulfur colloid dispersions are used as sentinel node detection. After injection the nanoparticles are taken up by the lymphatic system. This uptake mechanism could also be used in the field of drug delivery systems. The aim of our studies to perform basic investigations into the potential of inorganic colloidal particles as drug carriers.

Methods: Sulfur colloids were prepared by condensation of colloidal sulfur (method M1) or by proportionation of sodium sulfide and sodium sulfite in a solution containing sulfuric acid (M2). The formed particles were used as reference. Caffeine was added during the particle formation process (M2). PCS (photon correlation spectroscopy) measurements were performed (Malvern Autosizer IIc). SEM (scanning electron microscopy) (Cam Scan, Electron Optics Ltd) was used to visualize the nanoparticles, which had been collected on 0.1 µm filters (polyether sulfonate). The caffeine concentration was measured by HPLC (Agilent 1050, Analytik Jena).

Results: Sulfur nanoparticles in colloidal range were formed. The size distribution was not small according to PCS results. The particles were stable enough to be collected on filters and examined using SEM. The formation of the sulfur nanoparticles was influenced by the presence of the model drug caffeine. Particles with a mean diameter of 73 ± 6 nm were measured (PCS, n=8). The polydispersity index is smaller compared to the value calculated for drug-free sulfur nanoparticles. The nanoparticle dispersions containing caffeine were further investigated (n=8). By using filters (0.45µm and 0.1 µm) fractions of nanoparticles were separated, the remaining particle dispersions were investigated. About 4.7 % (w/w) of the total caffeine amount was assigned to the colloidal fraction. To get information if caffeine interacts with the surface of the sulfur particles, caffeine was added to colloidal sulfur. Even after 2 hours, the absorption of drug was negligible.

Conclusions: The presence of the model drug caffeine has an influence on the particle formation of sulfur nanoparticles, which are manufactured by proportionation. Surface adsorption as mechanism of interaction between caffeine and sulfur nanoparticles is not relevant.

The Unexpected Hidden Face Of The Cephalosporin Antibiotic Ceftazidime: From Biological To Chemical And Physical Activities Against OXidative Species Produced By Phagocytes

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Background: Overall several decades the fight against gram negative bacteria infections is a main challenge, and cetazidime (CAZ), a cephalosporin's family compound, is largely used in intensive care units to treat severe sepsis. Here in, we demonstrated that, beside its antibiotic effect, CAZ has unexpected antioxidant properties, and tried to better understand its mechanism of action.

Methods: Five in vitro (cell-free systems) and ex-vivo experimental models were designed to assess the capability of CAZ to protect 1) the brain (1 µg/ml) inhibitory activity of alpha-macroglobulin (M) submitted to an oxidant inactivation by stimulated phagocytes (PMNs) 2) human endothelial cells in culture against the toxicity induced by myeloperoxidase (MPO) or stimulated PMNs 3) alveolar cells (AS49, 10⁵ cells/ml. adherent or in suspension) against the oxidant damage induced by anoxia/reoxygenation, using oxymetry and EPR-spin trapping techniques 4) isolated from lipoperoxidation induced by irradiation, Fe²⁺/ASC system or ferryl species; 5) against hydroxyl radical or singlet oxygen (O²-) produced respectively by the Fenton (Fe²⁺/H₂O₂) and the Mallet (Fe²⁺/ascorbate system) or ferryl species; 6) 5) against hydroxyl radical or singlet oxygen (O²-) produced respectively by the Fenton (Fe²⁺/H₂O₂) and the Mallet (Fe²⁺/ascorbate system or ferryl species; 7) oxia/reoxygenation, using oxymetry and EPR-spin trapping techniques. 4) isolated from lipoperoxidation induced by irradiation, Fe²⁺/ASC system or ferryl species; 5) against hydroxyl radical or singlet oxygen (O²-) produced respectively by the Fenton (Fe²⁺/H₂O₂) and the Mallet (Fe²⁺/ascorbate system) or ferryl species; 6) against iron, and attenuation coefficients of excised tumor tissues were found to be dependent on the treatment regimen. The estimated rate of rise and equilibrium temperature, and the characteristic time to reach equilibrium are given for each group. Results obtained indicate that tumor growth decreases with increasing of ultrasonic intensity and sonication time. Tumor growth was delayed 4 to 6 days by combined treatment of 5-fluorouracil and ultrasound (US). Ultrastructural investigations of tumor cells showed several damages in cytoplasmic organelles and cytoplasmic vacuoles that increased with increasing ultrasonic intensity and sononation time. This damage appears as prominent crowded vacuoles among swollen ruptured organelles, chromatid fragments and severe increase in numbers of pyknotic and apoptotic cells.

Conclusions: The combination of 5-FU and ultrasound produced significantly greater antitumor activity than ultrasound or 5-FU alone.

Conclusions: The combination of 5-FU and ultrasound produced significantly greater antitumor activity than ultrasound or 5-FU alone.
A Novel Biomimetic Transformation To Construct Natural Product Frameworks
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The chemical synthesis of natural product and their derivatives has become an integrated part of developing pharmaceutical drugs. Their role is especially important in producing a scarce, but biologically intriguing natural product in larger quantities for further biological investigations and medicinal applications. During the last century, the synthetic approach has been striving for to apply the biogenetic consideration, aiming at the simulation of biogenetic key step which mimics nature in its elegance and efficiency. Such challenge has motivated our group to involve a biomimetic transformation in our synthetic plan of a potent anti-tumor agent hexacyclinic acid 1.

Central to the strategy is the biomimetic Michael-Prins reaction to construct the C ring and to deliver the tertiary alcohol, resulting from water addition to carbonyl ion intermediate.

Scheme 1. Biomimetic route utilizing Michael-Prins reaction in hexacyclinic acid 1 synthesis

Through our investigation, we were able to conduct the above reaction which provides not only a new C-C coupling reaction but also a Lewis acid-catalyzed reaction that could be carried out in aqueous media under a mild condition with a high catalyst recovery. This could give chance for pharmaceutical companies to synthesize efficiently and deliver more environmentally friendly reactions. 


Cell Penetrating Antibody Delivery To Intracellular Targets
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Background: A limitation of developing therapeutic antibodies specific for intracellular structures is to allow antibodies to enter cells without losing cell viability. The objective of this project was to create antibodies for intra-cellular targets to control cell proliferation.

Methods: Trans-membrane antibodies were generated by photo-affinity cross-linking a 17mer peptide derived from human sarcoma sarcoma virus. Targeting of antigens inside live cells is demonstrated by confocal microscopy, and functionally by inhibition of apoptosis.

Results: Trans-membrane penetrating (TMP) antibodies stained in live cells specifically actin and paxillin while naked antibodies did not. TMP antibodies also did not affect cell growth in culture. Furthermore, TMP modified anti-caspase-3 antibodies were shown to penetrate human T cell lymphoma cells and rapidly inhibit actinomycin D-induced apoptosis.

Conclusions: Membrane penetrating peptide-modified antibodies are endowed with a potency of targeting intra-cellular antigens in living cells and tissues, suggesting the clinical potential of immuno-therapeutic TMP-antibody delivery by cell-penetration.
Calcium Signaling And Angiogenesis
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Background: The proliferation and motility of vascular endothelial cells (EC) are critical steps in angiogenesis and are strictly controlled by different extracellular signals. Proangiogenic factors (VEGF, bFGF) generate cytosolic calcium rises through calcium entry from extracellular medium: this event is due to the opening of calcium-permeable channels in the plasma membrane, mainly activated by arachidonic acid (AA) and nitric oxide (NO). Even if some calcium entry blockers are under clinical trial at present with encouraging results, a better knowledge about the molecular nature of calcium channels and their intracellular regulation could lead to new and more specific strategies in therapeutic approach to cancer progression and angiogenesis.

Methods: Single cell calcium measurements by fluorescent probes, patch clamp, tubulogenesis assay, wound healing, time lapse, PCR, siRNA, proteomics.

Results: Here we describe proangiogenic intracellular calcium signals in endothelial cells derived from human breast carcinoma (B-TEC). AA, released upon EC stimulation with VEGF or FGF, promotes B-TEC proliferation, migration and organization of vessel-like structures in vitro. AA induces Ca²⁺ entry in the entire capillary-like structure during the early phases of tubulogenesis in vitro: no such responses are detectable in B-TECs organized in more structured tubules. An inhibitor of Ca²⁺ entry and angiogenesis, Carboxymethylidantrolae (CAI), significantly and specifically decreases AA-induced B-TECs tubulogenesis, as well as AA-induced Ca²⁺ signals in B-TECs. Finally, preliminary results suggest that at least part of AA-dependent calcium entry could be due to the opening of TRPV4, a well known Ca²⁺ entry channel.

Conclusions: 1) AA-activated Ca²⁺ entry is associated with the progression through the early phases of angiogenesis, mainly involving proliferation, motility and tubulogenesis. 2) It is downregulated during the neorenalization of tumor-derived endothelial cells in capillary-like structures. 2) inhibition of AA-induced Ca²⁺ entry may contribute to the antiangiogenic action of CAI.

1. Citrate release mechanism in prostate is electrogentic and K+-dependent citrate uptake in prostate cancer through intracellular high levels of Fe.

2. Citrate release mechanism in prostate is electrogentic and K+-dependent citrate uptake in prostate cancer through intracellular high levels of Fe.

3. Extracellular citrate in low concentrations can be taken-up by cancer synthesis. 4. Extracellular citrate in concentrations as low as 50 M was sufficient to increase prostate becomes metastatic.

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5. c-ACNT is overexpressed and upregulated in prostate cells resulted in the increased cell proliferation activity of MK was assayed by liquid chromatography analyses. The cell proliferation activity of MK was assayed using NIH3T3 cells.

Results: 1) Study 1: The ABIPRIME strips (designed to investigate the pseudomonal susceptibility/resistance), revealed that many psychrotrophs (ascribed to Pseudomonas, Stenotrophomonas, Acinetobacter, Burkholderia genera), besides exhibiting spoilage features were also multiresistant. Respectively, 42.3 %, 52.9 % and 94.1 % of the isolates, retrieved from farm, lorry and silos tanks, presented resistance to at least three classes of antibiotics (Microbial. Res. 162, (2007), 115-123).

2) Study 2: The isolates retrieved from milk, from conventional dairy farms, expressed higher prevalence of resistance to 4 of the 6 considered antibiotics; contrastly to gentamycin and trimethoprim-sulfamethoxazole, for which both categories of isolates presented similar resistance frequencies.

Conclusions: The observations from Study 1 suggest an accumulation of antibiotic multiresistant traits among psychrotrophs along the cold chain of raw milk storage and transportation. In Study 2, dairy management practices seem also to affect the level of susceptibility of psychrotrophs to antimicrobial agents. However, more milk samples have to be analysed to confirm this preliminary data. The question of whether psychrotrophs spoiling raw milk may play a role as reservoir for antibiotic resistance genes needs to be considered. In our recent studies, we showed at laboratory scale, that flushing raw milk with pure H2 gas constitutes an interesting perspective for limiting the spoilage and pathogenic potential of psychrotrophs; we believe the treatment may be of interest to control the antibiotic resistance potential of raw milk psychrotrophs as well.

Cytoskeletal and Adhesion Mechanisms of the Yeast in the Bacillus Subtilis System

citrate transport mechanism in prostate and its changes in malignant transformation. Implications for fatty acid synthesis in cancer

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Background: Prostate is a unique organ that produces, stores and releases large amounts of citrate. Excess citrate accumulation is possible because of a unique regulation of mitochondrial aconitase (m-ACNT). The level of citrate in prostatic lumen can reach 180 mM. Importantly, citrate level drops significantly when prostate becomes metastatic.

Methods: The present study was conducted on two human prostate cell lines: normal epithelial PNT2-C2 and strongly metastatic PC-3M. Citrate transport mechanisms were characterised using whole-cell patch clamp. Citrate metabolism in cancer cells was studied using spectrophotometric methods, real-time PCR and adhesion, motility and membrane secretory assays.

Results: Normal human prostate PNT2-C2 cells expressed a K+-dependent citrate transport mechanism designed primarily to release citrate into the lumen. It was electrogenic with the estimated stoichiometry of 1 citrate: 4 K+. Strongly metastatic prostate PC-3M cells were shown to express not only the same K+-dependent citrate release mechanism but also electrogenic, NAD+-dependent citrate uptake mechanism. Citrate preincubation of prostate cells resulted in the increased metastatic cell behaviour in cancer cells but had no effect on normal PNT2-C2. Extracellular citrate in concentrations as low as 50 M was sufficient to increase free fatty acids (FFA) synthesis in cancer cells. FA synthesis could be also decreased by (1) inhibiting fatty acid synthase (FAS) with cerulenin or (2) NADPH production through c-aconitase (c-ACNT) and isocitrate dehydrogenase (c-ICD) with oxaloacetic. Intracellular Fe chelator (inhibiting c-ACNT activity) also reduced FA synthesis.

Conclusions: 1. Citrate release mechanism in prostate is electrogenic and K+-dependent. 2. Prostate cancer cells express NAD+-dependent uptake mechanism. 3. Extra cellular citrate in low concentrations can be taken-up by cancer cells and increase their FA synthesis. 4. In cancer, RAFFP for FA synthesis is supplied mainly by c-ACNT/cICD. 5. c-ACNT is overexpressed and upregulated in cancer through intracellular high levels of Fe.
Pathogenesis Of Blood-Brain Barrier Breakdown Following Brain Injury: Windows For Therapeutic Intervention

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Breakdown of the blood-brain barrier (BBB) leading to cerebral edema is a life-threatening complication in many forms of brain injury such as infection, trauma, tumors and inflammation. In order for therapy to be effective in reducing morbidity and mortality, it must determine when to treat edema and with what agent(s). Increased endothelial caveolae leading to transcytosis of proteins and breakdown of tight junctions remain the principal mechanisms of BBB breakdown to plasma proteins leading to cerebral edema in brain injury. Our laboratory has characterized the time course of BBB breakdown in the rat cortical cold injury model. Our studies show that enhanced caveolae leading to transcytosis of protein occurs within minutes of the onset of brain injury and that this is associated with increased expression of caveolin-1, a major constituent of caveolae. Further, the caveolin-1 is phosphorylated in the vessels showing BBB breakdown. Altered expression of tight junction proteins follows a specific sequence with transient decreases in expression of junctional adhesion molecule (JAM) at day 0.5, and of claudin-5 at day 2 post-injury. Occludin expression is decreased throughout the period of observation starting at day 2. Therefore, therapy to control early brain edema should target caveolae and caveolin-1 while therapy to attenuate decreased expression of occludin can be administered within days of the onset of brain injury. Endothelial-specific growth factors are emerging as a strategy to treat cerebral edema. The potent anti-leakage effect of angiopeptin-1 has the potential to reverse early BBB breakdown in brain injury. (Supported by the Heart and Stroke Foundation of Ontario)

Influence Of The Etiology Of Liver Cirrhosis On The Response To Combined Intra-Arterial Chemotherapy In Patients With Advanced Hepatocellular Carcinoma

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BACKGROUND: We have previously reported that combined 24 hr intra-arterial chemotherapy (CIAC) protocols in patients with advanced HCC resulted in high rates of response in the 1st CIAC (Carcinoma Intra-Arterial Chemotherapy In Patients With Advanced Hepatocellular Carcinoma, 2004). However, whether the response to CIAC varies with the etiology of liver cirrhosis (LC) remains to be determined.

AIM: The aim of this study was to assess the influence of the etiology of LC on the efficacy of CIAC in advanced HCC.

METHODS: Fifty-two adult Japanese liver cirrhosis (LC) patients (45 men and 7 women) with a mean age of 63±16 years who were treated with CIAC between April 2000 and June 2004 at our hospital. All the patients had a JIS score of 3 or 4 (Kudo M. et al. Hepatology 40: 1396-1405, 2004). Their tumors were inoperable according to computed tomography findings. CIAC (LV at 12 mg/hr; CDDP at 10 mg/hr, and 5-FU at 250 mg/4 or 22 hr) was administered via the proper portal vein branches in the LC group and the C group. The CIAC regimen was continued for as long as possible.

RESULTS: There were 15 patients with HBV infection (B-LC group), 29 patients with HCV infection (C-LC group), and 8 patients with alcoholic cirrhosis (A-LC group). The Child-Pugh class was A for 6 out of 15 patients in the B-LC group and B for 9 patients, while the respective numbers were 14 and 15 patients in the C-LC group, 4 and 4 patients in the A-LC group. There were no patients with stage I disease, 7 patients with stage II disease, and 8 patients with stage III disease in the B-LC group, while the respective numbers were 4, 18, and 7 patients in the C-LC group and 2, 5, and 1 in the A-LC group. There was 1 patient with tumor thrombi involving major portal vein branches in the C-LC group and 2 patients in the A-LC group. The percentage of patients with a complete or partial response after 4 weeks of chemotherapy was 0% in the B-LC group versus 24% in the C-LC group and 25% in the A-LC group. The survival time of the A-LC and C-LC groups was significantly longer than that of the B-LC group, while the C-LC group was significantly higher after chemotherapy compared with that before chemotherapy (p<0.05). While the CIAC administered for the C-LC group was significantly higher after chemotherapy compared with that before and after chemotherapy in the A-LC group (p<0.05, Tukey's test). CONCLUSIONS: CIAC was more effective for AHC in patients with A-LC or C-LC compared with patients who had B-LC. Therefore, treatment for AHC should be selected according to the etiology of the underlying LC.

All abstracts are listed in alphabetical order of the presenting author.
**Influence Of Etiology On Host Immunity In Liver Cirrhosis Patients With Advanced Hepatocellular Carcinoma Treated By Combined Intra-Arterial Chemotherapy**


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**BACKGROUND:** We have reported that combined 24 h intra-arterial chemotherapy (CIC) improved the survival of patients with advanced HCC (sHCC) (World J Gastroenterol 2007 January 13;13(2):280-284). We have shown that this therapy was more effective for aHCC in HCV-related (C-LLC) or alcoholic (L-LLC) liver cirrhosis (LC) patients compared with HBV-related LC (B-LLC) patients before and after chemotherapy. We have also reported that Th2 cytokines are down-regulated in immune activation, while activation of type 1 T cells promotes antitumor immunity.

**AIM:** To estimate the influence of etiology on host immunity (Th1/Th2 balance) in LC patients with aHCC treated by CIC.

**PATIENTS/METHODS:** Thirty-one adult Japanese LC patients with aHCC were treated by CIC between 2005 and 2007 at our hospital. Their tumors were inoperable according to computed tomography findings. CIAC (LV at 12 mg/hr, CDDP at 10 mg/hr, and 5-FU at 250 mg/hr/hr) was delivered via the proper hepatic artery every 5 days for 4 weeks using a catheter connected to a subcutaneously implanted drug delivery system. The control group (C-LC) comprised of 13 adult Japanese healthy volunteers (HV). Blood samples were collected from the patients in the early morning before and after chemotherapy. Flow cytometry was used to assess cytokinetic IFN-gamma and IL-4 expression by peripheral blood CD4+ T cells, and the percentage of IFN-gamma+ and IL-4+ (Th1) or IFN-gamma- and IL-4+ (Th2) cells was calculated.

**RESULTS:** Eighteen of the 31 patients had C-LC, 7 patients had B-LC, and 6 patients had A-LC, and 6 patients had L-LLC in the C-LLC group. In the A-LC group, the percentage of Th2 cells before and after chemotherapy was 50.1% ± 10.2% and 59.9% ± 12.9%, respectively. The percentage of Th2 cells before and after chemotherapy was significantly higher than in the HV group before chemotherapy (p<0.05 by Tukey’s test). In the B-LC group, the percentage of Th2 cells before and after chemotherapy was significantly higher than in the HV group before chemotherapy (p<0.05 by Tukey’s test). There were no significant differences of Th1 cells before or after chemotherapy in the A-LC group compared with the HV group, although the percentage of Th2 cells before and after chemotherapy was significantly higher than in the HV group before chemotherapy (p<0.05 by Tukey’s test). There were no significant differences of Th1 and Th2 cells in the A-LC group compared with the HV group, although the percentage of Th2 cells before and after chemotherapy was significantly higher than in the HV group before chemotherapy (p<0.05 by Tukey’s test). In the B-LC group, there were no significant differences of Th1 cells before or after chemotherapy compared with the HV group, although the percentage of Th2 cells before and after chemotherapy was significantly higher than that in the HV group before chemotherapy (p<0.05 by Tukey’s test). There were no significant differences of Th1 cells before or after chemotherapy in the A-LC group compared with the HV group, although the percentage of Th2 cells before and after chemotherapy was significantly higher than in the HV group before chemotherapy (p<0.05 by Tukey’s test).

**CONCLUSIONS:** These results indicate that the present therapy was more effective for aHCC in A-LC patients with normal immune function and in C-LC patients without Th2 dominance than in B-LC patients with Th2 dominance before and after chemotherapy.

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**Stimulation Of Proline Uptake And Growth Of Escherichia Coli CSH4 And Its Mutants Under High Salinity Through Moderate Salinity Stress Treatment**

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**Background:** Moderate salinity stress (MSS) treatment was carried out for the activity stimulation of Escherichia coli and its mutants under high salinity. Aim: 1) To confirm the effectiveness of MSS treatment on proline uptake and growth 2) To compare the activity difference after MSS treatment among mutants with and without proline transporters 3) To identify the important factor in MSS solution to endure sufficient growth.

**Methods:** MSS treatment for E. coli CSH4 mutants was carried out in Davis minimal medium with s 5 mole of NaCl and 0.5 M NaCl at 30 °C for 1 h. E. coli CSH4 mutants used in this study were CSH4 (F' proP219 proU205), JT34 (CSH4 proP219 : Tn5), JT34 (CSH4 proU205 : Tn5), RM2 (CSH4 proP219 : Tn5) (proP219), WG207 (CSH4 proP219 : Tn5) (proU205), RG2 (CSH4 proP219 : Tn5) (proP219 : proU205), WG228 (CSH4 proP219 : proU205), WG227 (CSH4 proP219 : proU205), WG170 (CSH4 proP219 : proU205).

**Results:** Activities of proline uptake on proP strains (E. coli k-12, CSH4, JT34, JT31, RM2, WG207) were strongly enhanced after MSS treatment, but not for proP strains (WG170, WG227, WG228, WG170, WG207). Proline dehydrogenase encoded by putA supported proline uptake, i.e., amounts of proline in proP+putA strains were significantly lower than those of proP+ strains (K-12, CSH4, JT34) were higher than those of proP+putA strains (JT34, CSH4). PutA significantly inhibited cell growth considering the amount of proline accumulated in proP+putA strains (K-12, CSH4, JT34) were higher than those of proP+putA strains (JT34, CSH4).

**Conclusions:** 1) Proline uptake was enhanced for proP but not for proP strains by MSS treatment 2) Growth of mutants strains was dependent on the amount of proline accumulated during MSS treatment 3) Growth and proline uptake of proP+putA strains were K-1 dependent.
Amperometric Biosensor Development And Their Clinical Application

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Background: Polymamimes have important roles in biochemical processes. Their analysis in clinical diagnosis has substantial importance. In our earlier work an amperometric putrescine biosensor was successfully developed for clinical detection of bacterial infection. Elevated polyamine concentrations in human urine and serum appear to be associated with the presence of many types of cancer. The detection of diamines in clinical samples can be important for diagnosis of malignancy and for monitoring the efficiency of treatment. We made efforts to improve measuring range of the sensor.

Methods: Chemically modified electrodes and advanced electrochemical work stations were employed. Electrochemical method was used for preparation ultra thin size exclusion membrane to provided selectivity. Novel enzyme immobilization procedure was developed for getting highly active reaction layer.

Guinea pig blood and plasma samples served for spiking experiments. In vivo studies were also performed in abdominal tissue of anesthetized Wistar rats.

Results: A.) Improved biocompatible amperometric enzyme sensor was developed. It was made of an of a flat form amperometric microcell fabricated with thin film technology on flexible Kapton® substrate, and of an improved biocatalytic reaction layer. Highly active putrescine oxidase (PUDO) in reaction layer catalyzes the reaction:

\[
\text{Putrescine + H}_2\text{O} \rightarrow \text{H}_2\text{O}_2 + \text{NH}_4^+ + \text{H}_2\text{O}
\]

B.) A novel detection principle, named periodically interrupted amperometry (PIA) that generally can be applied for improving performance of amperometric biosensors has been developed. It involves application of new amperometric working program. Using it the sensitivity of amperometric biosensors like putrescine electrode could be improved by several orders of magnitudes.

Conclusions: Two different methods were successfully used for improving the performance of amperometric biosensors. The improvements were experimentally testing measuring low level putrescine that has importance in clinical diagnosis. The measuring procedure is simple, needs small sample size or in vivo application is available with minimal invasion.

Central Sympatholytic and Anti-arhythmic Effects of Serotonin-1A Agonists

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Background: Psychological stressors may provoke cardiac effects ranging from mild tachycardia to ventricular arrhythmias. Psychological stressors may also be seen in patients with coronary artery disease and ultimately to sudden cardiac death. At present, the only class of pharmacological agents used for preventing these consequences is β-blockers acting directly on the heart. Since these drugs have a number of side effects and counter-indications, the ability to suppress potentially deleterious increase in cardiac sympathetic activity at its origin, in the brain, would be a valuable alternative. In the current study, we tested whether ventricular arrhythmias precipitated by acute stresses could be suppressed by systemic administration of 8-OH-DPAT, a 5-HT1A agonist possessing central sympatholytic activity.

Methods: The study was conducted on 33 adult male rats instrumented for telemetry recordings of ECG, body temperature and locomotor activity. In the first experiment, rats were subjected to social defeat and were pre-treated with zatebradine (2 mg/kg sc.), a blocker of the pacemaker current.

Results: 8-OH-DPAT caused prolongation of basal RR interval (166±5 ms, p<0.01), increase in locomotion (105±152% counts/min, p<0.01) and hypothermia (37.9 to 36.6ºC, p<0.05). Subjecting vehicle-treated animals to social defeat caused shortening of RR interval, increase in locomotor activity and hypothermia, and provoked the occurrence of ectopic ventricular and supraventricular beats; all these effects were substantially attenuated by 8-OH-DPAT. Zatebradine caused prolongation of RR interval. In zatebradine/vehicle-treated rats, incidence of ventricular and supraventricular ectopic beats during social defeat increased 2.5-fold and 3.5-fold, respectively. 8-OH-DPAT administered after zatebradine completely abolished these stress-induced arrhythmias.

Conclusions: 1) Pharmacologically induced prolongation of RR interval results in an increased susceptibility to stress-induced cardiac arrhythmias, possibly due to the prolongation of the ventricular diastolic period with restored excitability; 2) Systemic administration of 8-OH-DPAT entirely abolishes these arrhythmic events, likely by suppressing stress-induced cardiac sympathetic outflow.
Aminoglycosides: Deadly Bullets In The Hands Of Unexperienced Therapeuticians

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Background: Therapeutic Drug Monitoring (TDM) of aminoglycosides has been a topic during the last thirty years. There is a tendency that – because of the once daily regimen – monitoring is considered not necessary anymore. Using the model we developed for efficacy and toxicity of aminoglycosides we are able to demonstrate that optimal dosing still needs a sophisticated system of TDM.

Methods: From a previous model we developed it appears that the efficacy of aminoglycosides is very sensitive to variation in the parameters of the model, for example the distribution volume (Vd) and the elimination rate constant (ke). The model predicts that this effect occurs when the drugs are given using suitably chosen parameters and that can be used in TDM. The extended mathematical model that is derived incorporates the effects of aminoglycosides on bacteria, the saturable and active uptake into kidney cells, the reversible nephrotoxicity and the irreversible ototoxicity. For a continuous administration, analytical solutions are calculated for the optimal concentration in the blood for efficacy and the concentration without nephrotoxicity.

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Conclusion: The model can be used to design appropriate antibiotic drug regimes to combine maximal efficacy and minimal (acceptable) toxicity.

Synthesis And Biological Evaluation Of Novel Pyrazole And Pyrazolopyrimidinyl Pyrazoles As Anti-Inflammatory Agents

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Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for the treatment of pain and inflammation. Their mechanism of action involves inhibition of prostaglandin synthesis by the enzyme cyclooxygenase (COX). It has been reported that pyrazolopyrimidines inhibit COX enzymes, and therefore, may possess anti-inflammatory properties. Recently, we have synthesized novel pyrazole and pyrazolopyrimidinyl derivatives to investigate their anti-inflammatory effects.

Methods: A diketo-dipyraclo compound and an acid chloride of pyrazol(3,4,5)-pyrazolopyrimidines as anti-inflammatory agents

Conclusion: The model can be used to design appropriate antibiotic drug regimes to combine maximal efficacy and minimal (acceptable) toxicity.

Abstracts

All abstracts are listed in alphabetical order of the presenting author.
Immunotherapy is a promising approach for the treatment of follicular lymphoma (FL), the most common low-grade B-cell non-Hodgkin’s lymphoma that is considered incurable and results in fatal outcome in the majority of patients. Immunization with customized vaccines made from the clonal tumor immunoglobulin molecule, termed idiotypic, induced tumor-specific T-cell immunity in greater than 80% of patients with lymphoma. However, objective clinical responses were observed in only a minority of patients following idiotypic vaccination. Although the magnitude or quality of the immune responses may be a reason for the low clinical response rate, resistance to the effector phase of the antitumor T-cell response due to immunosuppressive mechanisms in the tumor microenvironment is also thought to play a major role. Important negative regulatory pathways that inhibit T-cell function include extrinsic suppression by regulatory T cells, direct inhibition through inhibitory ligands such as PD-L1, and metabolic dysregulation of essential amino acids such as tryptophan. The relative contributions of these inhibitory processes will be reviewed and novel approaches will be discussed.

Levofloxacin For Typhoid Fever: An Unparalleled Local Success Story

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Fluoroquinolones especially Ciprofloxacin emerged as a drug of choice for treating typhoid fever during the last decade of the previous century while Levofloxacin acquired the prestigious title of Respiratory Quinolone due to its action by obtaining very high drug concentration both in the alveolar macrophages as well as lung tissue and its ability to destroy the Atypical Respiratory Pathogens. Because of a trend reported at the end of the previous century concerning decreased sensitivity of Salmonella typhi against ciprofloxacin the application to study hospitalized uncomplicated typhoid fever patients with oral levofloxacin was granted by the Faculty of Medicine University of Indonesia. This initial open study using 500 mg of levofloxacin once daily for 7 days consisted of 53 screened hospitalized typhoid fever patients enrolling 48 patients of the result in whom 31 cases were positive by either blood culture or polymerase chain reaction or by significant serological increase of titer. From these 31 cases one cases was later excluded because of a concomitant sinus infection. After the astonishing result of resolution of fever within 2.5 days with a minimum number of side effects which were all tolerable and without any clinical failure or S. typhi fecal carrier state one month post treatment, it was decided to continue the study and compare two regimens of treatment for uncomplicated typhoid fever. A random single blind comparative study of 500 mg ciprofloxacin twice daily for one week against once a day 500 mg levofloxacin also for one week was proposed that also received full support by the ethical committee of the Faculty.

Levamisole Resistance In Parasitic Nematodesinvestigated At The Molecular Level

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The nicotinic acetylcholine receptor (nACHR) is an important determinant of signal transmission at the neuromuscular junction. Helminth nACHRs are selectively targeted by many drugs such as the anthelmintic family avermectins (e.g., levamisole). However, the high efficacy of levamisole treatments against gastrointestinal parasitic nematodes of ruminants has been compromised by the development of resistance in field parasite populations. In order to investigate molecular mechanisms involved in levamisole resistance a candidate gene strategy has been initiated. In the free-living nematode Caenorhabditis elegans, the levamisole-sensitive nACHR is composed of five multi-transmembrane spanning subunits encoded by unc-29, unc-1, unc-63, unc-38 and lev-8 genes and mutants lacking one of those genes are resistant to levamisole. Here we have identified and sequenced unc-29, lev-1, unc-63 and unc-38 orthologs isolated from the trichostrongylid nematode Haemonchus contortus that is causing major economic losses to sheep industry throughout the world. Expression studies of those genes in levamisole resistant and susceptible isolates of H. contortus revealed specific expression of alternatively spliced RNA messenger in resistant isolates. If the alternative splicing of nACHR subunits is well documented in insects, this work constitutes to our knowledge the first report of such a phenomenon in nematodes.
Longterm Therapy Of Brain Tumors With Temozolomide: Review Of Tolerability And Efficacy In 53 Patients

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Background: Temozolomide (TZM) is a 2nd generation alkylating agent with significant efficacy for low-grade and malignant brain tumors. The drug is administered orally for 5 days every month (150-200 mg/m^2/day), using a conventional schedule. Due to TZM's excellent tolerability and lack of cumulative systemic toxicity, some patients are receiving treatment for 12 to 24 months. The efficacy, safety, and tolerability of this longterm therapeutic approach remains unclear.

Methods: We performed a retrospective chart review of all Neuro-Oncology Center patients who had undergone temozolomide chemotherapy for 12 months or longer.

Results: A total of 53 patients (median age 45 years) met the criteria; tumor types included glioblastoma multiforme (GBM; 17), oligodendroglioma (10), anaplastic glioma (12), astrocytoma (6), other glioma (7), and CNS primary lymphoma (1). Forty-one patients had received irradiation; 10 had prior chemotherapy. The median number of monthly TZM cycles was 20 (range 12-28; 38 patients > 18 cycles), with a median TZM dose of 400 mg/day. Median time to progression was 34+ months (range 14 to 65+ months; 30+ months in GBM cohort), with 15 objective responses by MRI (28.3%). Toxicity included mild to moderate fatigue (47%), and thrombocytopenia (47%). Of 1087 total cycles of TZM, 18 (1.7%) were scored as toxicities.

Conclusions: Longterm treatment with TZM is feasible, and demonstrates durable activity and acceptable toxicity in patients with gliomas, including GBM.

In Vitro Pharmacodynamic Evaluation Of Intracellular Activity Of Antibiotics (ABs) Alone Or In Combination Against A Small Colony Variant (SCV) Of Staphylococcus Aureus

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Background: SCVs show reduced AB susceptibility and persist intracellularly, which may cause therapeutic failures. The intracellular activities of OXA (oxacillin), PA ( fusidic acid), CL1 (clindamycin), GEN (gentamicin), RIF (rifampin), VAN (vancomycin), LNZ (linezolid), Q-D (quinupristin-dalfopristin), DAP (daptomycin), TSC (teicoplanin), MXF (moxifloxacin), TLV (tigecycline), and ORI (oritavancin), alone or in combination, were examined in THP-1 macrophages infected by a stable thymine-dependent SCV in comparison with normal phenotype and revertant isogenic strains isolated from the same cystic fibrosis patient.

Methods: Intracellular activities were determined in THP-1 macrophages after 24 h or 72 h of exposure to ABs. Combinations were tested at fixed concentrations and then using the Fractional Maximal Effect method (FME).

Results: At C_{m80}, ORI caused a 2 log CFU reduction at 24 h, RIF, MXF, and Q-D, a similar reduction at 72 h. All other ABs showed a static effect at 24 h and 1 log CFU reduction at 72 h. Dose-effect studies showed a bimodal curve with 2 successive plateaus at 0.4 and 3.1 log CFU for ORI; maximal effects of 1.1 to 1.7 log CFU for TSC, MXF, and RIF, and of ≤ 0.6 log CFU for the other ABs. Addition of thymidine restored the SCV intracellular growth, but did not modify the toxic activity except for Q-D. All drugs showed strong intracellular activity against normal or revertant phenotypes than against SCVs, except TSC and ORI.

Conclusion: Intracellular SCV are poorly susceptible to most ABs, which may contribute to the difficulty of eradicating such infections. Our studies may help in selecting most active drugs or appropriate combinations to rationalize AB treatment of persistent infections involving SCVs.

Doripenem, A New Carbapenem: Optimizing Dose To Treat Increasingly Resistant Gram-Negative Pathogens.

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Background: The growing number of infections due to multi-drug resistant (MDR) Gram-negative pathogens such as Pseudomonas aeruginosa has prompted exploration for new, highly effective anti-infective agents as well as the more rational use of available anti-infectives. Doripenem is a new carbapenem with increased in vitro microbiologic potency, low potential for seizure induction, and exploration for new, highly effective anti-infective agents as well as the more longterm therapy of brain tumors with temozolomide: review of tolerability and efficacy in 53 patients.

Methods: These features allow for increased dose and prolonged infusion duration, which optimizes the carbapenem time-dependent pharmacokinetics and pharmacodynamics (PK/PD), and hopefully increases clinical efficacy against serious Gram-negative infections while minimizing the emergence of resistance.

Results: Doripenem has been studied at 500 mg infused over 1 hour (Q1H) for the treatment of complicated urinary tract infections, intraabdominal infections, and nosocomial pneumonia. A prolonged infusion of the 500 mg dose over 4 hours has been studied in later-onset ventilator-associated pneumonia, acknowledging that pathogens with higher carbapenem MICs are more likely in this patient population. Additional studies are underway with a 1-gam dose infused over 4 hours in patients with nosocomial pneumonia, including ventilator-associated pneumonia, who are at particular risk of carbapenem resistant P. aeruginosa infection. Exploring the safety of high dose doripenem and the longer stability in solution, strategies to increase the time over MIC of this highly potent carbapenem, theoretically allows for the treatment of infections due imipenem-resistant pathogens where other therapeutic options are severely limited.

Conclusions: In summary, doripenem holds promise as a new "magic bullet" for the treatment of infections involving MDR Gram-negative pathogens, particularly Pseudomonas aeruginosa.

Authors’ disclosure statement: Susan C. Nicholson and Janet A. Peterson are employees of Ortho-McNeil Janssen Scientific Affairs, LLC.

Sugammadex A Novel Cyclodextrin For The Reversal Of Neuromuscular Blockade

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Background: Following surgery, reversal of the non-depolarizing effects on skeletal muscle relaxation is facilitated by the administration of an acetylcholinesterase inhibitor. Although effective, this method of reversal presents several practical issues including muscarinic adverse effects and administration timing. Sugammadex is a modified gamma-cyclodextrin compound pharmacologically unrelated to the acetylcholinesterase inhibitors. Currently, pharmaceutical uses of cyclodextrins have been primarily limited to excipients in drug formulation. Sugammadex is a selective relaxant binding agent, which forms a 1:1 complex with steroidal non-depolarizing neuromuscular blockers within the plasma. This results in lowering the availability of the neuromuscular blocker at the nicotinic receptor. Unlike acetylcholinesterase inhibitors, prior studies have demonstrated that sugammadex can elicit rapid reversal during profound neuromuscular block.

Methods: Using acceleromyography, we describe reversal in two surgical cases requiring pharmacologic reversal of neuromuscular blockade. The primary outcome measure was a train of four ratio (TOF) of 0.9. In the first case, the acetylcholinesterase inhibitor neostigmine antagonized the effects of non-depolarizing blockade with rocuronium and vecuronium. In a subsequent case, sugammadex was administered for reversal of rocuronium-induced neuromuscular blockade.

Results: Both methods employed for reversal were effective. The TOF ratio of 0.9 was achieved 4 min, 41 sec after administration of neostigmine following partial spontaneous recovery of TOF ratio (0.5) and 1 min, 14 sec with sugammadex following partial spontaneous recovery of TOF ratio (0.25). Additionally, a TOF ratio of 0.9 was achieved in the neostigmine-treated patient, there was a period of 4 min, 15 sec when the ratio decreased to below the desired level of 0.9. In contrast, the TOF ratio was maintained at 0.9 or higher in the sugammadex-treated patient, once a level of 0.9 was obtained.

Conclusions: Sugammadex provides novel approach for the reversal of amino- steroidal induced neuromuscular blockade. In contrast to acetylcholinesterase inhibitor, sugammadex does not increase endogenous levels of acetylcholine or require the co-administration of anticholinergic agents.

Authors’ disclosure statement: The authors and the Mayo Clinic College of Medicine have conducted sponsored clinical research studies of sugammadex for Organeri Schering Plough.
Hormone-Immunotherapy Significantly Prolongs Clinical Benefit And Median Overall Survival Of Metastatic Endocrine Dependent Breast Cancer Patients

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Background: Metastatic disease from breast cancer is incurable. In endocrine-dependent patients, antiestrogens are commonly administered as first and second line therapy. Regrettably, tumour growth becomes resistant to this relatively well tolerated therapy. In the past, beta-interferon was added to tamoxifen increased their mutual anti-tumour activities. Nevertheless, no effective clinical application has been developed. We started an exploratory clinical trial based on the association of these immunostimulating cytokines with antiestrogens for first line salvage therapy of hormone dependent metastatic breast cancer.

Methods: Thirty three consecutive breast cancer patients with distant metastases, most of them with involvement of multiple organs, were studied for responsiveness to treatment with first-line salvage anti-estrogen therapy, combined with beta-interferon and interleukin-2 immunotherapy. Clinical response and survival were compared with that of 30 consecutive historical control patients treated with anti-estrogen therapy alone.

Results: Controls showed, as expected, a median duration of response, a median survival time after treatment, and after diagnosis of distant metastases, of 16, 31 and 34 months, respectively. After a median follow-up of 74 ± 39 months (range 24-209) from the beginning of first line antiestrogen salvage therapy, the interval times in the studied patients were 33 (p<0.001), 74 (p<0.001) and 79 (p<0.001) months. One long-term survivor appeared to be cured after 155 months from the beginning of first line antiestrogen therapy.

Conclusion: These data suggest that immunotherapy, given in an outpatient setting in addition to conventional antiestrogen salvage therapy, is very well tolerated and provides an important benefit in endocrine-dependent metastatic breast cancer.
Cryomunnoimmunology Introduced After Reimplantation Of Malignant Bone Tumor Treated With Liquid Nitrogen

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Background: 1) In the laboratory, systemic antitumor responses following reimplantation of tumors treated with liquid nitrogen-cooled dendritic cells were evaluated by measuring immune reactions and counting distant metastases in mice after coronary stenting. Clinical results showed that interaction with liquid nitrogen-cooled dendritic cells were increased after coronary stenting and that distant metastases were measured after reconstruction with liquid nitrogen-treated autogenous grafts.

Methods: 1) LPS mouse osteosarcoma cells were cultured subcutaneously into C3H mice. After two weeks, tumors were treated with a) excision (E) (n=15); b) liquid nitrogen (LN) (n=15); or c) liquid nitrogen plus dendritic cells (DCs) (n=15). Four days after treatment, IFN levels, LDH levels, T-lymphocyte counts in lung metastases were measured. 2) IFN and IL-12 levels were measured in blood samples collected before surgery and one and three months after surgery from 23 patients with malignant bone tumors treated with tumor-bearing autografts frozen by liquid nitrogen.

Results: IFN (in IU/ml) was significantly higher in DCs (118.29 ± 5.71; p=0.01) than in both LN (37.22 pg/ml ± 2.74) and E (8.25 ± 2.76). LN was significantly higher than E (p = 0.03). Mean metastasis area was significantly smaller in DCs (53.38 ± 1.46) p < 0.01) than in both LN (132.41 ± 2.59) and Tx (24.12 ± 3.60); LN was significantly smaller than E (p < 0.01). Mean number of CD8+ T-lymphocytes was significantly higher in DCs (82.3 ± 2.56) (P < 0.05) than in both LN (24.6 cells/mm2 ± 0.57) and E (0.64 ± 0.56). LN was significantly higher than E (p < 0.01). 2) Mean IFN-γ relative concentration of one month after and three months after autograft before surgery were 149.7 and 268.3%, and mean IL-12 relative concentration were 170 and 432.2%, respectively. Composite values for patients showed progressive increases in IFN-γ and IL-12 levels one and three months after surgery.

Conclusions: 1) Treatment of tumor tissue with liquid nitrogen can activate the immune system and inhibit metastatic tumor growth. Dendritic cells enhanced immunological activity synergistically. 2) Patients with malignant bone tumors who received liquid nitrogen treated with liquid nitrogen showed evidence of immune system activation. 3) These responses suggest that liquid nitrogen treatment of bone tumors may offer certain unique benefits.

Effect Of Insulin Resistance On In-Stent Restenosis After Coronary Stenting In Type 2 Diabetic Patients

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Background: Metabolic syndrome is related to cardiovascular diseases. Insulin resistance is one of causes for metabolic syndrome. The aim of this study was to elucidate the mechanism of the effect of insulin resistance on in-stent restenosis after coronary stenting in type 2 diabetic patients.

Methods: A prospective, randomized trial, involving 54 type 2 diabetic patients referred for coronary stenting who were randomly assigned to either the control or the pioglitazone group, was performed. Quantitative coronary angiography was performed at study entry and at six months follow-up. Endothelial nitric oxide synthase (eNOS), tumor necrosis factor alpha, interleukin-6, leptin, and adiponectin were measured at study entry and at six months follow-up.

Results: 28 patients were randomly assigned in the control group and 26 patients were assigned in the pioglitazone group. There were no significant differences in glyceric control levels or in lipid levels in the two groups at baseline or at follow up. Insulin, homestasis model assessment insulin resistance, eNOS, and leptin at follow up were significantly reduced in the pioglitazone group compared with in the control group. The late luminal loss and in-stent necrosis were significantly less in the pioglitazone group than in the control group. Multiple regression analysis showed that leptin independently correlated with late luminal loss. Conclusion: The treatment with pioglitazone in type 2 diabetic patients significantly reduced leptin. This decreased leptin improved insulin resistance and endothelial function with the reduction of insulin. The improved endothelial function affected the reduction of in-stent restenosis.
Combined Therapy With Pitavastatin And Eicosapentaenoic Acid Improve Platelet Activation Markers In Hyperlipidemic Patients With Type 2 Diabetes Mellitus

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Background: Platelet-derived microparticles (PDMP) play an important role in the pathogenesis of diabetic vasculopathy, and statins or eicosapentaenoic acid (EPA) have been shown to have a beneficial effect on atherosclerosis in hyperlipidemic individuals. We previously showed that pitavastatin and EPA, when administered in combination, downregulated PDMP in hyperlipidemic patients.

Methods: In this study, patients with type 2 diabetes mellitus were randomly assigned to one of four groups: (1) pitavastatin, (2) EPA, (3) pitavastatin plus EPA, and (4) placebo. PDMP levels were assessed in platelet-rich plasma from whole blood using flow cytometry.

Results: PDMP levels were significantly lower in the pitavastatin plus EPA group compared to the other groups. Additionally, the expression of CD40 ligand and PDMP was decreased in the pitavastatin plus EPA group.

Conclusions: These results suggest that combined therapy with pitavastatin and EPA may be effective for the prevention of vascular complication in hyperlipidemic patients with type 2 diabetes.
LC-MS/MS Strategies For Increased Metabolite Coverage In Metabolomics

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Background: Metabolomics is the global and untargeted measurement of as many metabolites as possible in a given biological system. There is no one single analytical technique available to measure all the metabolites in a complex system such as a cell, however, liquid chromatography mass spectrometry (LC-MS) is establishing itself as the preferred metabolomics analytical technique. We have explored the possibility to use ultra high pressure chromatography and multiple ionization strategies for the mass spectrometry analysis in order to expand the number of detectable metabolites.

Methods: Methanolic extracts of human serum was used to investigate what the impact of various LC-MS strategies had on the number of detected metabolites. Evaluation of chromatography was performed through comparison of 1.7 and 3.5 μm particle size columns. Evaluation of the multiple ionization strategy was performed using an Agilent MSD SL system with ESI and Multimode ionization sources. All LC-MS data was extracted and aligned using the freely available XCMS software (http://mMasspec.scripps.edu/xcms/xcms.php)

Results: Ultra high pressure liquid chromatography (UPLC) resulted in 20% more ion features detected when compared to conventional HPLC. Performing MS analysis in both positive and negative ionmode doubled the number of unique ion features compared to positive mode only, and performing APCI (atmospheric pressure chemical ionization) on the same sample resulted in an additional 20% increase in unique ion features.

Conclusions: To achieve truly non-targeted and global metabolite analysis of a complex biological system, multiple analytical techniques are needed. We have shown that sub 2μm particle size liquid chromatography paired with multiple ionization mass spectrometry can dramatically increase the number of measured metabolites.

Predicting Fluoroquinolones Ability To Kill Resistant Streptococcus Pneumoniae Isolates Expressing Different Genetic Mutations: Target Attainment Analysis Simulating Therapeutic Doses To Patients With Community Acquired Pneumonia

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Background: Streptococcal pneumonia is a major cause of morbidity and mortality worldwide. Fluoroquinolones are one of the mainstay drugs for treatment of these infections. However emerging resistance poses a threat to the class’s future utility. This work aims to evaluate the probable efficacy of ciprofloxacin, levofloxacin, gemifloxacin, garenoxacin, and moxifloxacin in eradicating infections and preventing continued growth of resistance.

Methods: Using patient data from strep pneumonia patients in hospitals and MIC data from the CROSS study, drug regimens were compared to see the likelihood of attaining AUIC50/MIC ratios depicting goal clinical outcomes.

Results: Probability of target attainment of the fluoroquinolones against all the genetically identified resistant isolates of Streptococcus pneumoniae is shown in the following table:

Table: 

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<tr>
<th>Drug</th>
<th>Dose</th>
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<td>Cip (S)</td>
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<td>Cip (ELF)</td>
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<td>Gare (S)</td>
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<td>Gare (ELF)</td>
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Conclusions: Very few regimens are able to prevent further growth of resistant organisms when Pneumococcal mutations have occurred. Only garenoxacin and moxifloxacin were able to eradicate extremely resistant isolates in serum and ELF respectively.

Unexpected Effects On Angiogenesis By Type Of Low-Molecular-Weight Heparin (LMWH) And By Type Of Vehicle In Chemotherapy

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Background: Tumor growth is angiogenesis dependent. The study of anti-angiogenic effects per se in tumors is not feasible, however. We have developed a mammalian non-tumor angiogenesis assay allowing the objective quantitative assessment of relevant variables following s.c./p.o./i.v. treatment with test agents. Aim: 1) To study the effect of i) standard heparin (UH), ii) different mean molecular weights of UFH, (iii) fractions of UFH, (iv) intratumoral and intravenous injections of different MWs (produced by heparinase digestion of UFH), and (v) dalteparin (produced by nitrous acid depolymerization of UFH). 2) To examine how low-dose metronomic-like chemotherapy affects angiogenesis induced by VEGF-A, a key heparin-binding angiogenic factor in most tumors.

Methods: The mesentry assay in adult rats was used: a pro-angiogenic agent was injected i.p. and the ensuing angiogenesis in the mesenteric window was recorded following the s.c. injection of a heparin or the s.c. continuous infusion chemotherapy. Heparin: UH (MW =15 kD), MW 2.5, 8, 15 and 22 kD fractions of UFH, MW 2.5 and 5.5 kD tinzaparins, and MW 6.0 kD dalteparin. Cytostatic: cyclophosphamide, paclitaxel, doxorubicin, cisplatin, and 5-FU. Conventional vehicles were used, some containing a radical oxygen species (ROS) scavenger. In other cases the ROS scavenger N-acetylcysteine (NAC) was given concurrently with the chemotherapy.

Results: Heparin MW influenced endothelin-induced angiogenesis strictly (r = 0.97) low MW inhibited and high MW stimulated. In VEGF-A-mediated angiogenesis, the 2.5 kD tinzaparin fraction lacked effect while the 5.5 kD tinzaparin fraction inhibited angiogenesis. Conversely, dalteparin (MW 6.0 kD) stimulate angiogenesis. Cisplatin and 5-FU monotherapy surprisingly stimulated angiogenesis. NAC monotherapy was inert. 5-FU + NAC co-treatment reversed the significant pro-angiogenic effect of 5-FU into a significant anti-angiogenic effect. The anti-angiogenic effect of paclitaxel emerges only when the vehicle contained a ROS scavenger.

Conclusions: 1) Heparins exert MW-related effects in angiogenesis. 2) Similarly sized LMWHs, produced by diverse methods, affect angiogenesis differently. 3) Metronomic-like chemotherapy drug-specifically influences angiogenesis: some drugs stimulate while other inhibit angiogenesis. 4) ROS play a major role in angiogenesis during low-dose chemotherapy

Apoptin Magics: Bullet Or Sensor?

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Background: Apoptin, a protein derived from chicken anemia virus (CAV), induces selectively apoptosis in human tumor and transformed cells as compared to normal cells. Various gene-therapy approaches have shown the power of apoptin as a tumor-selective killer. Apoptin interaction with e.g. the anaphase promoting complex results in tumor-selective apoptosis. Furthermore, apoptin becomes phosphorylated selectively in tumor cells by a yet unknown kinase activity. These features make apoptin both a potential bullet for novel anti-cancer therapy and a sensor for identifying novel potential drug targets.

Methods: We used the transforming ability of the SV40 large T (ST) oncogenic proteins in normal human fibroblasts to identify tumor-related pathways sensed by apoptin by co-transfection experiments. Besides, we asked whether protein-transduction domain (PTD4)-mediated delivery of apoptin protein becomes phosphorylated selectively in tumor cells.

Results: Very few regimens are able to prevent further growth of resistant organisms when PneC mutations have occurred. Only garenoxacin and moxifloxacin were able to eradicate extremely resistant isolates in serum and ELF respectively.

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Neurodevelopment Of Children Following Exposure To Psychotropic Medications During Gestation: A Novel Approach To Behavioral Teratology

NULMAN I

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Background: Maternal psychopathology should be balanced against the potential teratogenic effects of psychotropic medications (PTMs), higher levels of stress hormones, neonatal syndromes, higher fetal central nervous system (CNS) transmitter activity, and child’s long-term neurodevelopment. Assessing neurocognitive development of children exposed to medications in pregnancy is an inseparable part of drug safety studies in teratology, which are complicated by multiple confounders. Study design plays an integral role in controlling these factors.

Objectives: 1. To review available literature and determine the possible risks of adverse fetal/child CNS development following exposure to PTMs in utero.
2. To present a novel approach to studying behavioural teratology, while controlling for genetic and environmental factors by testing unexposed siblings from the same families.

Methods: A systematic review of studies on long-term child neurodevelopment was conducted. IQ scores of children exposed to Venlafaxine (VLF) (n=32) were compared to their unexposed VLF siblings and healthy controls. Participants were hierarchically matched for age, gender and order of delivery. The primary outcome measure is the children’s full scale IQ measured by the WPPSI-III Scales of Intelligence. Statistical analysis accounted for the clustering effect.

Results: Full Scale IQ, Performance IQ and Verbal IQ were not different between the VLF-exposed children and their unexposed siblings (105±12 vs100±8; 102±15 vs105±7; 105±12 vs95±10). Healthy controls scored significantly higher than the VLF group in all three IQ measures (P = 0.011; 0.041; and 0.028 respectively). There were no differences between the groups in maternal IQ, socioeconomic status or children’s physical characteristics.

Conclusions: In utero exposure to PTMs was not associated with impaired long-term neurocognitive development. Assessment of siblings helps to separate the impact of antidepressant drugs from genetic and environmental factors and is strong evidence in psychotropic drug safety studies. Factors other than the antidepressant are strongly associated with children’s cognitive abilities. Supported by Wyeth Pharmaceuticals.
Improving Treatment Of Urinary Tract Infections In Elderly: Roles And Functions Of Information Technologies To Suggest Antibiotic Prescriptions And To Measure Their Effects

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Background: The evolution of Escherichia coli’s resistances and the development of ESBLs call for a better transmission of experts’ knowledge to practising physicians; information technologies can contribute to this purpose.

Methods: To conceive Electronic Antimicrobial Drug Aid Regulation 1) Guidelines adapted to elderly are given to allow a dynamic and interactive consultation. 2) Therapy by antibiotic failures are recorded: they are related either to intolerance or to the occurrence of resistance; broad spectrum molecules prescriptions linked to patients’ details are also recorded. 3) An instrument panel is designed for the follow-up of E. coli sensitivities: in a short stay unit, comparison is made by a chi-square test of 2 series including 283 and 303 colonies, each one corresponding to 12 months of urine sampling respectively in 2006 and 2008.

Results: 1) Suggestion of antibiotic on the screen, the practitioner determines his diagnosis with signs of gravity and checks for the existence of complications: in case of emergency, a probabilistic treatment is suggested. When bacteriological data are available, suggested antibiotics are divided into 5 classes and developed into 7 tables according to the seat and gravity of the infection and various types of complications: oral or injectable form, dose and posology, length of treatment are specified, renal function is taken into account. 2) In case of adverse drug event, the imputability of the antibiotic is evaluated according to Naranjo’s method; if resistance occurs, one records supporting factors related to the patient and those related to care practices. 3) The instrument panel shows a significant increase of resistance to the amoxicillin – ac. clav. (p < 0.05); a sensitive, yet not significant, increase of resistance to acid nalidixic (25.44% versus 32.67%) and ESBLs (2.47% versus 5.61%).

Conclusions: The computer program should make it possible to reduce unnecessary antibiotic prescriptions especially in case of asymptomatic bacteriuria, to deliver an effective treatment in case of severe infection, to prevent adverse drug events and to attenuate ecological risk linked to the increase of bacterial resistances.

A Computationally Designed Mutant Of The Metallo-β-Lactamase IMP-1 Exhibits Enhanced Catalytic Efficiency

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Background: Metallo-β-lactamases (MBLs) inactivate a broad range of β-lactam antibiotics, they can be transferred horizontally, and there are no clinically useful MBL inhibitors. In addition, the evolutionary potential of MBLs is of great concern. They can be transferred horizontally, and there are no clinically useful MBL inhibitors. In addition, the evolutionary potential of MBLs is of great concern.

Methods: Computational protein design was employed to predict improved variants of the MBL IMP-1. The predictions were validated through biochemical characterization and kinetic analysis of purified enzymes overexpressed in E. coli. Subsequently, molecular dynamics simulations of enzyme-substrate intermediate complexes were used to decipher the molecular mechanisms for enhanced catalytic efficiency.

Results: Two mutations (F218Y and S262A) were computationally designed in IMP-1. Experimental validation showed that the single mutant IMP-1-F218Y was superior to the wild-type enzyme IMP-1: overexpression in E. coli yielded a higher amount of soluble protein, the protein folded well and was thermally stable, as monitored by circular dichroism spectroscopy, and it exhibited enhanced catalytic efficiency toward the following β-lactams: nitrocefin, cephalothin, cefotaxime, cefazidime, benzylpenicillin, ampicillin, and imipenem, mostly due to a decreased K<sub>m</sub>. Multiple molecular dynamics simulations showed that the F218Y mutation leads to an altered hydrogen bonding pattern and to a movement of a β-hairpin loop toward the active site, which could account for the decreased K<sub>m</sub>. Conclusions: 1) The IMP-1-F218Y mutant was successfully predicted to be a more efficient enzyme than the wild-type enzyme IMP-1. 2) The enzyme could easily be overexpressed through a one-nucleotide change and could lead to enhanced antibiotic resistance. 3) Successful prediction of improved MBL variants could assist the design of better antibiotics and MBL inhibitors.

Kinetic Properties Of Recombinant Factor Vila (fVil)a And The Complexity Relating These To Treatment Response

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Background: Haemophilia A (HA) and B (HB) are inherited coagulation disorders caused by deficiency of factor VIII (FVIII) or IX (FIX). These patients are treated with replacement therapy with FVIII or FIX. Approx. 30% of HA<sup>1</sup> and 5% of HB<sup>2</sup> patients develop inhibitors, making replacement therapy extremely difficult. Human subjects may have a higher incidence of resistance to acid nalidixic (25.44% versus 32.67%) and ESBLs (2.47% versus 5.61%).

Methods: RNA was extracted from 104 tumour and normal breast specimens, as well as from pre-surgery serum specimens from four recently diagnosed breast cancer patients, from their breast tumour/matched normal biopsies, and from serum specimens procured approx. 3 months post-surgery. Serum specimens from healthy age-matched volunteers acted as controls. RNA from each specimen was examined using U133 Plus2.0 arrays. Following normalisation, statistical filters were applied to identify significant differentially-expressed (DE) genes. Using univariate and multivariate methods, individual mRNAs were extensively investigated for associations with patients’ clinicopathological characteristics. qRT-PCR was used to validate microarray data.

Results: mRNAs were detected in all specimens. Overall, approx. 8% (of 54,675 probesets representing transcripts on the microarray) were present in serum and approx. 45% were detected in breast tissue. 7448 transcripts were DE (P=0.0058) between tumour and normal breast specimens and 998 (P=0.0009) and 1369 (P=0.0013), respectively, between those that resulted in recurrence or death within 5 years compared to those that did not. Clinical statistical analysis identified 36 mRNAs as potential novel biomarkers; some of which tended to be associated with tumours of a basal-like profile. qRT-PCR analysis of 5 transcripts randomly selected from these validated our microarray results.

Conclusions: The implication of these novel findings is that, using microarrays, it may be possible to identify panels of intracellular and extracellular biomarkers that are useful diagnostic, prognostic and/or predictive of outcome for cancer patients.

Acknowledgements: HEGA’s PRULU, Dublin City University’s Research Fellowship and Ireland’s Health Research Board.
Development of Gelatin-Based Nanoparticulate Formulation for the Delivery of Human Insulin

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Background: The design of systems for oral delivery of proteins is in fact a real challenge and very little work has been reported in this field of research. However, the tendency of proteins to denature and lose activity poses severe limits to the reactions that can be performed on the carrier, ranging from purification, and storage of the delivery system. Given the above background, we developed a nanoparticulate formulation of insulin under gentle nanoprecipitation conditions.

Methods: Insulin was dissolved in water (pH 7.0) by means of a sonicator. A concentrated alcohol solution (15% w/v) was added slowly to the insulin solution while stirring with a magnetic stirrer to produce an insulin-gelatin dispersion. The dispersion was centrifuged, and the supernatant was lyophilized. Formulations were characterized by size, zeta-potential, and insulin content. The in vitro stability of the insulin-loaded nanoparticles was assessed by subjecting them to acidic pH and elevated temperature conditions.

Results: The nanoparticles were spherical, with a mean diameter of 90 nm and a polydispersity index of 0.2. The zeta-potential of the nanoparticles was -30 mV, indicating good stability. The in vitro release of insulin from the nanoparticles was less than 5% after 24 h, indicating good stability.

Conclusions: The developed formulation of insulin-loaded nanoparticles is suitable for oral delivery of insulin, providing a stable and controlled release of the insulin.

Screening Of Antioxidants For Inhibitory Activity Against Lung Metastasis Of Murine Colon Cancer Cells In Mice

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Background: Cancer metastasis is a major cause of death in cancer patients. Thus, it is important to find promising agents with antimetastatic activity. A variety of antioxidants such as antioxidative vitamins and polyphenols have recently been proposed to be effective in inhibiting cancer metastasis mainly based on the inhibition of tumor invasion, growth and experimental lung metastasis. Here we report the antimetastatic activity of ceftriaxone sodium-loaded mucoadhesive gelatin microspheres and a promising agent was further examined on anti-tumor immune cells.

Methods: Experimental lung metastasis was induced by intraperitoneal inoculation of murine colon 26-L5 cells through a tail vein of mice and tumor nodules on the surface of lungs were determined. Test compounds were administered intraperitoneally 5 consecutive days starting from the 1st day to mice scheduled to receive a dose of 1 micromol. Cell invasion and growth were evaluated by transwell chambers and WST-1 solution, respectively. Free radical scavenging assay was carried out using the stable radical, 1,1-diphenyl-2-picrylhydrazyl. In some mice, experiments, mice receiving anti-asialoGM1 or 2-chloroadenosine, or nude mice were used to examine roles of natural killer (NK) cells, macrophages, or T cells, respectively, in the antimetastatic effect of a test compound.

Results: Among 20 compounds tested, epigallocatechin gallate (EGCG) exhibited most significant reduction by 77% in tumor metastasis. EGCG also inhibited the dose-dependently with 98% suppression at 2 micromol. Statistically significant inhibition was not observed by other compounds. These results indicate the potential of EGCG in the growth inhibiting activities of test compounds and their inhibition rates of tumor metastasis. We have next examined roles of anti-tumor immunity in the EGCG's effect, and found that depletion of NK cells completely abolished the effect, whereas a marginal reduction of the effect was seen in nude mice and macrophage-depleted mice.

Conclusions: These results suggest that EGCG has potential benefit for tumor metastasis inhibition. Its antimetastatic effect may be mediated mainly through NK cell activity.

Abstracts

All abstracts are listed in alphabetical order of the presenting author.
Radiotherapy And Multi-Agent Chemotherapy (Procarbazine, ACNU And Vincristine) For High-Grade Gliomas: A Prospective Study

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Purpose: To prospectively evaluate the efficacy and toxicity of radiotherapy combined with multi-agent chemotherapy using procarbazine, nimustine (ACNU) and vincristine in adults with high-grade gliomas.

Materials and Methods: Patients aged 18 years or older with a histologically confirmed supratentorial glioblastoma or grade 3 gliomas (anaplastic astrocytoma, anaplastic oligoastrocytoma and anaplastic oligodendroglioma) were enrolled. Radiotherapy protocols were hyperfractionated radiotherapy (HF-RT) or radiotherapy immediately after hyperbaric oxygenation (HBO) (HBO-RT). The protocol of HF-RT consisted of 2 fractions per day of 1.2 Gy to a total dose of 72 Gy (10 fractions per week). The protocol of HBO-RT consisted of daily 2 Gy fractions for 5 consecutive days per week up to a total dose of 60 Gy, with each fraction administered immediately after HBO with the period of time from completion of decompression to irradiation being less than 15 minutes. Chemotherapy consisted of procarbazine (90 mg/m² orally, days 1 to 14), ACNU (85 mg/m² intravenously, day 1) and vincristine (0.5 mg/m² intravenously, days 1 and 8) and was administered during and after radiotherapy, up to a maximum total of 4 courses.

Results: Between 1997 and 2003, a total of 51 patients (36 patients with glioblastoma and 15 patients with grade 3 gliomas) were enrolled. HF-RT was administered in 10 patients treated between 1997 and 1999, and HBO-RT was administered in 41 patients treated between 2000 and 2003. All 51 patients were able to complete a planned radiotherapy using HF-RT or HBO-RT with 1 course of concurrent chemotherapy. Of 38 assessable patients, 20 (52%) had an objective response including 6 CR and 14 PR. The median survival time in all 51 patients and 36 glioblastoma patients was 17.5 months and 15.5 months, respectively. On univariate analysis, histologic grade (p < 0.001) and Karnofsky performance status (p = 0.01) had a significant impact on survival, and on multivariate analysis, histologic grade alone was a significant prognostic factor for survival (p < 0.001). Although grade 4 leukopenia and grade 4 thrombocytopenia occurred in 10% and 8% of all patients respectively, these were transient with no patients developing neutropenic fever or intracranial hemorrhage. No serious non-hematological or late toxicities were seen.

Conclusions: These results indicated that radiotherapy with multi-agent chemotherapy (procarbazine, ACNU and vincristine) was effective and safe with virtually no late toxicity in patients with high-grade gliomas.

A Novel Polymorphic Purine Complex At The 1.5 kb Upstream Region Of The Human Caveolin-1 Gene And Risk Of Alzheimer's Disease; Extra-Short Alleles And Accumulated Allele Homozygosity

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Crucial interaction of caveolin-1 (CAV1) with b- and g-secretases, and aberrant expression of the gene encoding this protein in Alzheimer's disease (AD) support a role for CAV1 in the pathophysiology of this disease. We report a novel polymorphic purine complex stretching ~150 bp of genomic DNA at the 1.5 kb upstream region of the human CAV1 gene, alleles and genotypes of which are associated with sporadic late-onset AD. Extra-short alleles were observed in the case group that were absent in the control subjects. Incomplete homozygosity for haplotypes was also observed at this region in the Alzheimer's cases (p<0.002). This region contains GGAA and GAAA motifs, the consensus binding sites for the C/EBP and IRF family transcription factors, respectively, and is highly conserved in distantly-related non-human primates with respect to location and motif sequence. The effect of this complex sequence on the expression of CAV1, and the related mechanisms in the pathophysiology of AD remain to be clarified.

Translating Drugs As Magic Bullets: A New Approach To Drug Discovery

OHLSON S

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A plethora of weak/transient biological interactions (association constant: Kd > μM), either working alone or in concert, occurs frequently throughout biological systems. We are beginning to realize their importance in complex biological networks for the support of life. This appreciation has important implications in for example drug discovery as we can question the current paradigm of drug design to find the highest possible binders (drugs) to a given target (receptor). Development of transient drugs as magic bullets, defined by their weak binding to target, can be based on high-off-rates, multivalent approaches or multiple targets. Now, high throughput techniques are available to discover such drug candidates and diverse molecular libraries are available. The greatest problem yet to overcome is maybe the mind-set of the individual researcher that weak/transient binders are undesired and therefore of no benefit.

I will introduce you to the basic concepts of transient binding and their role in biological systems, define and discuss benefits of a transient drug, show potential screening procedures to find them, speculate about possible targets for transient drugs including cardiovascular diseases, CNS disorders and pain conditions.
Development Of Highly Anti-HIV Active And Locally Toxic 4’-C-Ethynyl-2’-Deoxy-2-Fluoroadenosine And A Proposal Of The Way To Develop Highly Active And Locally Toxic Antiviral Modified Nucleosides

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Background: The existing antiretroviral therapy has several critical problems such as (i) the emergence of new drug-resistant HIV-1 mutants, (ii) the need to take large dosages of drugs, and (iii) drug side effects. In order to solve these problems three working hypotheses, (i) the way to prevent the emergence of resistant HIV-1 mutants against nucleoside reverse transcriptase inhibitors, (ii) the way to decrease the toxicity of nucleosides, and (iii) the way to provide nucleosides with stability to both enzymatic and acidic degradation, were proposed in our study.

Methods: Based on the working hypotheses, many kinds of 4’-C-substituted nucleosides were synthesized and evaluated for their biological activity and stability to both enzymatic and acidic degradation. Its deoxy-2-fluoroadenosine(two positions modified 2’-deoxyadenosine derivative), which is highly potent against all HIV-1s, does not have acute mouse toxicity, is stable to intracellular enzymatic catalysis and acidic degradation, its triphosphate has a very long intracellular half-life, does not greatly inhibit mitochondrial DNA polymerase γ.

Conclusions: 1) 4’-C-ethynyl-2’-deoxy-2-fluoroadenosine which is highly active against all HIV-1s and low-toxicity was developed. 2) The proposed three working hypotheses were generally applied to the development of new highly active and low-toxic antiviral modified nucleosides. 3) The working hypotheses were proved to be valid. 4) The working hypotheses could be further improved to develop highly active and low-toxic antiviral modified nucleosides, especially those against HIV-2 virus because flu-virus uses RNA-dependent RNA polymerase like as HCV does.

Authors’ disclosure statement: Merck’s scientists developed a highly anti-HCV active and low-toxicity 4’-C-methyl-7-fluoroadenosine which is a two positions modified riboside derivative.

Our study and Merck’s study showed that human DNA and RNA polymerases are more sophisticated than viral polymerases in the point that they scarcely absorb the nucleosides modified at two or more positions of physiologic nucleosides into their active centers but viral polymerases do accept them, and further suggested that by taking advantage of the difference it will be possible to develop highly active and low-toxic antiviral modified nucleosides, especially those against Flu-virus because Flu-virus uses RNA-dependent RNA polymerase like as HCV does.
Is The Induction Chemotherapy Response- And Recurrence Rate Depend On N0 Or N+ Stage In Oral Squamous Cell Cancer? 

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Background: In Hungary the morbidity and mortality rate of head-neck cancer have increased in the last 30 years. One of the major causes is the high rate of primary recurrences after surgery. In this study the authors correlated the regression and recurrence of N0 and N+ cases following chemotherapy.

Methods: During a 6 year period, 90 consecutive treated OSCC patients were entered into this retrospective study. From these patients 39 were N0 and 51 were N+ stages. Twenty-five patients received Bleomycin- Vincristin- Methotrexate (BVM), twenty BVM+ Cisplatin and forty-five BVM +Mitolactol neo adjuvant chemotherapy. After three courses of chemotherapy the progression (CR,PR,NR) and side effect rate were determined. All of the patients were operated and observed the number and localisation of recurrences in three years follow-up time.

Results: The NO cases came from T2-3, while N+ from T2-4. The regression were in NO group CR:46%, PR:54%, but in N+ group CR:12%, PR:74%, NR:14%. The side effects were slight (grade I-II) and reversible. The recurrence rate for NO group was 15% (lymph node metastasis), while for N+ group 59% (primary or metastasis). The recurrence rate was only 4% for complete responders. The three year tumor-free survival for NO group 85%, N+ group 43%. 

Conclusions: After neoadjuvant chemotherapy there was very good chance for tumor free survival in complete-remission from N0-N+ stages and partial responders coming from N0 stage. For these cases (45 patients) the recurrence rate was 13%. The N+ was bad for full responders or no-responders coming from N+ stage (45 patients) as the recurrence rate was 70%.

High Uptake Of Vaccines – A “Magic Bullet” In Control The Burden Of Hospitalisation Attributable To Childhood Mumps And Rotavirus Infections 

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Background: The Polish immunisation program consists of two vaccination systems; the routine (obligatory) and recommended (optional) vaccination. The routine vaccinations (mumps) are bought by Polish National Health Fund and recommended vaccinations (rotavirus [RV]) are paid by individuals. Aim: To assess the impact of different immunisation systems on the hospitalisation burden attributable to vaccine-preventable mumps and RV infections in children between 2003 and 2008, in north-eastern Poland.

Methods: A retrospective analysis of hospital discharge data obtained between January 9, 2003 and June 30, 2008, at University Children’s Hospital in Białystok. Electronic hospital discharge data were reviewed to select records of children 0-18 years of age with ICD-10 codes for RV gastroenteritis (RVGE – A08.0) and for mumps (B26.0; B26.1; B26.2; B26.9). The nosocomial RV infections in 2008 were analysed based on the Hospital Infection Control Team database. The results were compared with mumps vaccines uptake (web database of the National Institute of Hygiene in Warsaw) and local RV vaccines uptake.

Results: Of 5213 children hospitalised during study period 2003-2007, 626 (12%) were discharged as RVGE, and 319 (6.1%) as mumps infection. The proportion of hospitalisations attributable to the mumps infection declined from 15.5% in 2003 to 0.2% in 2007, in line with increasing mumps vaccination uptake. In the year 2003, 39% of Polish children have been vaccinated against mumps whereas in 2007 – 97%. In the first 6 months of 2008 no case of mumps was hospitalised.

The percentages of hospitalised cases attributable to RVGE increased during the study period from 7.7% in 2003 to 18.1% in 2007. In the half of 2008, the percentage of hospitalised RVGE cases reached the highest level (39.7%). In 2008 year, the percentage of nosocomial RV cases among total RV cases was estimated to be 16.1%. In 2007, 89 infants under 6 months of age out of 1997 children born in our region were vaccinated against RV (vaccine coverage – 3%).

Conclusion: Routine vaccination system in line with high uptake of vaccines are needed to provide herd immunity and to control the burden of hospitalisation.
Yoshioka M

To examine whether nicotinic acetylcholine receptor (nAChR) antagonist could suppress impulsive behavior in rats. To determine the relationship between the estimated dose of nicotine intake per day and impulsive behavior in male Wistar-strain rats (10-13 weeks old). In human study, delay and probability discounting task was employed to assess impulsivity. In animal studies, 3-choice serial reaction time task was used to assess impulsivity and the effects of nicotine were tested. Moreover, nAChR antagonist suppressed impulsive behavior.

Methods: 3-ketoacyl-CoA thiolase (3-KAT) was determined by RT-PCR. Oxidation in diabetic rats. Expression of 3-KAT with its substrates needed which is currently performed in our laboratory. The finding along with the increase in 3-KAT expression suggests that higher enzyme activity may suggest that the inefficient therapy of human malignant melanoma can be connected with up-regulation of 3-KAT.

Conclusions:

1. Diabetes increased the rates of fatty acid oxidation that was accompanied by deteriorated cardiac function 2-3) The inhibitory effect of TMZ on cardiac mechanical function and fatty acid oxidation was not detected at 0.8 mM palmitate in the perfusate. This finding may suggest that the inefficient therapy of human malignant melanoma can be connected with up-regulation of 3-KAT.

Results:

1. Diabetes increased the rates of fatty acid oxidation that was accompanied by deteriorated cardiac function 2-3) The inhibitory effect of TMZ on cardiac mechanical function and fatty acid oxidation was not detected at 0.8 mM palmitate in the perfusate. This finding may suggest that the inefficient therapy of human malignant melanoma can be connected with up-regulation of 3-KAT.
25-Years Of Sight Saving By Preventing Postoperative Scarring With Local Use Of Antimetabolites

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Background: Chronic glaucoma is a progressive blinding disease. A major risk factor of disease progression is an increased intraocular pressure (IOP). When topical and laser treatments are inadequate, surgical treatment is required. Intraocular pressure can be reduced by IOP-lowering surgical activities, such as filtering surgery. Following our understanding of both that MMC toxic effects relate to inhibition of neighboring cell proliferation, and are mild and temporary, MMC, the more potent agent, is toxic also to quiescent neighboring cells. It use could be risky, termed “time-bomb”. Gradual thinning of the postoperative conjunctival roof of the new outflow path, called the filtering bleb, led in many eyes to spontaneous ocular perforation and intraocular infection (endophthalmitis). Nowadays, following our understanding of both that MMC toxic mechanism and the modification of the trabeculotomy-MMC approach, MMC may be used safely.

Methods: Since the introduction of local application of antimetabolites in 1982, initially 5-fluorouracil (5-FU) by us and later mitomycin C (MMC) by others, surgical success rate in all groups commonly exceeds 90%. As an inhibitor of fibroblasts proliferation 5-FU was injected under the conjunctiva twice daily for 14 days after surgery. Following our observations that it is also toxic to existing fibroblasts, few injections are administered and only when imminent scarring is evident. 5-FU side-effects relate to inhibition of neighboring cell proliferation, and are mild and temporary. MMC, the more potent agent, is toxic also to quiescent neighboring cells. It use could be risky, termed “time-bomb”. Gradual thinning of the postoperative conjunctival roof of the new outflow path, called the filtering bleb, led in many eyes to spontaneous ocular perforation and intraocular infection (endophthalmitis). Nowadays, following our understanding of both that MMC toxic mechanism and the modification of the trabeculotomy-MMC approach, MMC may be used safely.

Conclusions: 1) The local administration of MMC orMMC intraoperatively and/or postoperatively became the turning point that improved glaucoma prognosis substantially during the last decade. This led to an extensive increase in the number of glaucoma operations, that saved sight of many thousands worldwide. 2) The local use of chemotherapeutic agents has spread to avoid failure of other surgical approaches, such as ocular pterygium and limbal-path operations, as well as in fields other than ophthalmology, such as the use of a coated cardiac stent.
Interactions Between NE And EAAT

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Background: Excitatory Amino Acid Transporters (EAAT) are the main mechanism for its removal from the synaptic space. We have reported that alpha-1 antagonism (prazosin) prevents cocaine sensitization and it is accompanied by marked changes in Glutamate transmission (Ortiz et al 2006). We examined possible changes in EAAT activity brought about by cocaine exposure.

Methods: Rat cortex (CTX) and prefrontal cortex (PFC) slices were incubated with different agents for at least one hour prior to measuring EAAT activity. The slices were then incubated with 50 µM 1H[3]Glutamate for 10 minutes. The media was then removed and the slices washed twice. The slices were then solubilized in 50 µL of 0.5 M NaOH overnight. The radioactivity was quantified after acidification with 50 µL of glacial acetic acid and addition of 1 mL Ethanol using a Beckman LS 1800 scintillation counter.

Results: Fifty micromolar NE increases EAAT activity in PFC but not in cortical slices. Cocaine increases EAAT activity in PFC slices but not cortical slices. Prazosin also increases EAAT activity, however, propranolol (beta blocker) had no effect, nor did it prevent the effects of NE.

Conclusions: 1) Our results show modulation of EAAT activity by NE and 2) suggest non-alpha, non-beta mechanism(s).

Applied Reverse Vaccinology: A Meningococcal Serogroup B Vaccine For Infants

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Background and Aims: Although strains of serogroup B N.meningitidis are the predominant disease causing strains in many regions of the world today, there is no truly global vaccine available to prevent this particular meningococcal infection. In a technique known as “reverse vaccinology,” potential vaccine candidates were predicted by analyzing the entire genome sequence of an invasive MenB strain. Through genetic engineering and from the investigation of 350 potential antigens, surface proteins were identified that best induced an antibody response. Building on this genomics approach, it was possible to develop a vaccine that offered protection against multiple strains of MenB. Phase II trials have demonstrated satisfactory safety, tolerability and immunogenicity. In addition, rMenB is the first recombinant MenB protein vaccine to induce an immune response in infants.

Methods: Safety and immunogenicity of the rMenB vaccine was assessed in a 2, 4 and 6 month schedule. The immunogenicity was measured by serum bactericidal assay using human complement (HBsA).

Results: The trial demonstrated satisfactory safety, tolerability and immunogenicity. Local and systemic reactions of the vaccine candidate were similar in frequency and intensity to routine infant immunisations with the exception of fever. A moderate, short-lasting temperature rise not exceeding 39.0°C following the first dose was reported more frequently in the rMenB arm than in the control. Preliminary analysis shows 89% (44/76-SL, ST32), 96% (5/99, ST8) and 85% (N238/254, ST41/44) hSBA ≥1:4 post 3rd dose against three serogroup B strains representing the major vaccine antigens. The majority of disease causing strains worldwide express at least one of MenB antigens.

Conclusions: rMenB vaccine is well tolerated and immunogenic against a panel of serogroup B strains in young infants when administered in a three dose schedule two months apart. This vaccine is entering phase 3 clinical trials.
Complex Carbohydrate-Based Cancer Vaccines: Magic Bullets in The Making?

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1Organic Synthesis Core Laboratory, 1The Laboratory for Biogorganic Chemistry, Molecular Pharmacology and Chemistry Program, 2Laboratory of Tumor Vaccinology, Clinical Immunology Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York; 3Department of Chemistry, Columbia University, Havemeyer Hall, New York.

Background: A hallmark of cancer establishment is heterogeneity within a single patient and even within a single organ. Heterogeneity of cancer manifests itself in many ways. In addition to all disrupted signaling pathways, the complex carbohydrates that are displayed at the cell membrane are also severely disrupted. Some are overexpressed in certain cancers more than others, while others are not properly processed. Challenging the immune system with these aberrant carbohydrate motifs seems to be a promising strategy. Vaccination against cancer though deprived of glory to date, has provided more than was originally expected of it.

Methods: Over the past decade, several major developments in chemical synthesis and manipulation of complex carbohydrates have made these complex structures available in pure single forms. This has re-opened the door to the systematic study and improvement of carbohydrate based cancer vaccines. So far vaccination against cancer was tested in late stages of disease when everything else failed, and when patient’s immune system is equally compromised. In the many vaccination trials as a means of “preventive measure against cancer relapse” complex carbohydrates have proven efficacious in prolonging patients’ life compared to untreated patients.

Results: Challenging the immune system with a unimolecular multipotent synthesized carbohydrate-based cancer vaccine produced an antibody count that is superior to the sum of its epitopes used together as a mixture.

Conclusions: Fluorquinolones provide higher levels of ocular antibiotic penetration, with moxifloxacin providing the highest levels in the aqueous humor (p<0.01). Dosing moxifloxacin every 15 minutes for one hour prior to collection results in aqueous humor concentrations more than 3x the MIC of fluorquinolone resistant staphylococci (2.0 µg/mL) and more than 100x the MIC of fluorquinolone sensitive staphylococci (0.05 µg/mL).

Race Disparity In Stroke Risk Factors: The Berlin–Ibadan Experience; A Retrospective Study

OWOLABI MO1, PLATZ T2

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Background: Different workers have reported racial disparities in the distribution of risk factors for stroke and stroke subtype (ischemic vs hemorrhagic). No transcultural translational studies have been conducted to confirm and relate these disparities to one another. Our objective was to identify differences in the distribution of risk factors for stroke and stroke subtypes among urban-dwelling stroke patients in Nigeria, a developing country, and Germany, an industrialized country.

Methods: Consecutive stroke patients in Ibadan (100) and Berlin (103) were studied. Their hospital records were screened to identify documented vascular risk factors and stroke subtype.

Results: The stroke patients in Ibadan were younger than those in Berlin (t = 4.940, P = 0.000). Hypertension was significantly more common in Ibadan than in Berlin while cigarette smoking, dyslipidemia, atherosclerosis, and cardiac factors were significantly more frequent in Berlin. Cerebral infarction was more common in Berlin (80%) than in Ibadan (63%).

Conclusion: The risk factors associated with cerebral infarction were more frequent in Berlin. We suspect that racial disparity in risk factors for stroke may account for the difference in proportions of stroke subtype in black and white populations. Larger prospective community-based multinational transracial studies are required to confirm these disparities and identify possible underlying genetic, dietary, and socio-economic factors.

The Ocular Penetration Of Antibiotics Using A Rabbit Model That Emulates Human Topical Dosing.

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Alcon Research Ltd., Fort Worth, TX, USA.

Background: Various antibiotics are being used topically to treat ocular infections. We have developed a new rabbit model that emulates human topical ocular dosing. The model gives a level of precision that is superior to both human and animal pharmacokinetic studies, and the results correlate well with human aqueous humor levels obtained during cataract surgery. The model has been used to explore the ocular penetration and distribution of a number of commercial ophthalmic antibiotics.

Methods: Anesthetized rabbits were given a single topical dose (50 µL) of the various commercial antibiotics followed by a 30 minute controlled period that emulates the human eye with manual blinking (4 blinks/min) and a supplementary tear flow (2 µL/min). Tear samples (1 µL) were collected at 2 minute intervals throughout the dosing. The rabbits were euthanized after 60 minutes and ocular tissue samples were collected. In another study, a more frequent dosing regimen was explored. All tissue samples were extracted by sonication in water, and antibiotic concentrations were quantified using HPLC.

Results: The tear concentrations of the antibiotics decreased at a first-order rate and generally were below detectable levels after 20 minutes. The tissue levels for some of the antibiotics evaluated, 60 minutes after dosing, are shown in the table:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Moxifloxacin</th>
<th>Levofloxacin</th>
<th>Gatifloxacin</th>
<th>Tobramycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosing</td>
<td>Single</td>
<td>4 times</td>
<td>Single</td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>2.2 ± 0.4</td>
<td>7 ± 1</td>
<td>0.40 ± 0.07</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>2.6 ± 0.1</td>
<td>16 ± 3</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Cornea</td>
<td>7 ± 3</td>
<td>32 ± 1</td>
<td>3.7 ± 0.6</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Iris-ciliary body</td>
<td>1.4 ± 0.3</td>
<td>6 ± 1</td>
<td>0.37 ± 0.02</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Sclera</td>
<td>0.7 ± 0.2</td>
<td>3 ± 2</td>
<td>0.8 ± 0.7</td>
<td>1.0 ± 0.3</td>
</tr>
</tbody>
</table>

Conclusions: Cytokine Profiles Of Patients With Cutaneous Leishmaniasis

OZBILGE H, KAYA E

University of Einyes, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Kayseri, Turkey.

Background: The Leishmania spp. are obligate intracellular protozoa that cause a spectrum of diseases, including cutaneous, mucocutaneous, and visceral leishmaniasis, in tropical or subtropical countries. Sainliura is an area highly endemic for cutaneous leishmaniasis caused by the protozoan Leishmania tropica and it has been for many years an important focus in Southern Anatolia of Turkey. This research was planned to detect the cytokines in sera of patients with active cutaneous leishmaniasis in Sainliura.

Methods: In this study, the cytokine measurements were made in sera of 25 patients with active cutaneous leishmaniasis, before and after the sodium stibogluconate therapy and 25 healthy control people. Cytokines such as IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10 and IL-12 were measured in groups and the results were compared.

Results: IL-2, IL-4, IL-6, IL-10, IFN-γ levels were found higher in active cutaneous leishmaniasis than control group, while no differences in IL-12 levels were found. IL-5, IL-6, IL-10, IFN-γ levels were found lower at post treatment compared to pretreated patients with cutaneous leishmaniasis.

Conclusion: Following up the cytokine levels of these patients with cutaneous leishmaniasis can give an idea on the course of the disease and can contribute to schematize cytokine treatment.

All abstracts are listed in alphabetical order of the presenting author.
A Molecular Perspective On Integron-Associated And Transferable Antibiotic Resistance In Clinical And Aquatic Isolates Of Gram-Negative Bacteria In Northern Region Of Turkey

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Background: We analyzed the data obtained from our earlier studies concerning the mobile genetic elements such as conjugative resistance plasmids and class 1 and class 2 integrons in multi-drug resistant Gram-negative pathogens isolated from different aquatic or clinical environments in the northern region of Turkey.

Methods: Disk diffusion and agar dilution methods were used in antibiotic susceptibility testing. Transferable antibiotic resistance was detected by conjugation assays. Class 1 and class 2 integron gene cassettes were screened and then described by sequencing. Sequences were compared to the current GenBank databases using the BLAST suite of programs. CLUSTALW amino acid sequence alignments were produced for comparison.

Results: Resistance to ampicillin, tetracycline, streptomycin, trimethoprim and sulphamethoxazole was commonly high in multi-drug resistant organisms from both aquatic and clinical isolates, and much of them were transferable traits carried on conjugative plasmids. The gene cassettes such as the families of aadA and dfrA inserted in integrons were commonly shared between aquatic and clinical isolates, and some of them were novel gene cassette arrays and new alleles.

Conclusions: We conclude that further molecular epidemiological investigations should be committed to monitor for these antibiotic resistance genes in various environments in the other regions of Turkey to take precautions for slowing down the progression of antibiotic resistance evolution country-wide.
Circulating epithelial tumor cells (CETC) allow gene analysis of the residual tumor burden, individual chemosensitivity testing and monitoring of the adjuvant setting in a curative

PACHMANN K# and PACHMANN U

1Clinic for Internal Medicine II University Hospital Friedrich Schiller University Jena Germany and 1Center for Transfusion Medicine Bayreuth Germany

Background: Although most malignant tumors have no detectable metastases at diagnosis, most patients do not die from the primary tumor but from metastases during the subsequent course of disease. Circulating epithelial tumor cells (CETC) emanating from the primary tumor are assumed to be the source of such metastases. Understanding the composition and the genetic variability of CETCs, chemosensitivity of remnant CETCs before and after specific therapies and monitoring during systemic therapy will further our understanding of therapy response and resistance.

Methods: Monitoring of neoadjuvant systemic therapies included 50 lung cancer patients and 75 breast cancer patients, of adjuvant therapies 200 breast cancer patients, and chemosensitivity testing has been performed in over 100 patients. CETCs were detected and monitored from peripheral blood of cancer patients requiring not more than 1 ml of blood by staining with fluorescein labeled antibodies to EpCAM and CD45 antigen by laser scanning cytometry or automated image analysis. Analysis for genetic aberrations was performed by strong fluorescence in situ hybridization and single cell PCR and in vitro cytotoxicity testing by analysis of specific tumor cell killing by chemotherapeutic agents.

Results: Visible epithelial cells, suspect of tumor origin, were detectable in more than 95% of tumor patients with a good correlation between tumor size and the number of CETCs in sera from the hepatocytes of the liver. These predictions were compared to measured in vivo uptake, permeation, binding and metabolism. Parameters from the hepatocyte model to estimate the ratio of intracellular to extracellular metabolite, and the kinetic behavior of statins in the liver will lead to a better understanding of the pharmacokinetics, pharmacodynamics and potential drug-drug interactions of statins.

Conclusion: The study included the drugs atorvastatin, cerivastatin and lovastatin and the evaluation of their free concentrations in serum and the hepatocytes of the liver. The study showed that the free concentrations of statins in the liver were greater than the free concentrations in serum and that the free concentrations of statins are relevant for the inhibition of HMG-CoA reductase enzyme. The results are consistent with the previous findings and support the hypothesis that the free concentrations of statins in the liver are higher than the free concentrations in serum.

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2University of Michigan, Ann Arbor, MI, 48109, USA and 3Fred Hutchinson Cancer Research Center, Seattle, WA, 98103, USA

Background: No validated biomarkers exist for acute graft versus host disease (GVHD), the major complication of allogeneic hematopoietic cell transplantation.

Methods: We screened plasma with antibody microparticles for 120 proteins in a discovery set of 42 transplant patients. We then measured the levels of the best biomarkers from the discovery set by sequential ELISA to create a composite biomarker panel that we tested in samples from 424 transplant patients randomly divided into training (n = 282) and validation (n = 142) sets.

Results: Analysis of 23 proteins in the discovery set revealed eight potential biomarkers. Logistic regression analysis of these eight proteins in the samples of the training set determined a composite biomarker panel of four proteins (interleukin-2-receptor-alpha, tumor-necrosis-factor-receptor-1, interleukin-8, and hepatocyte growth factor) that optimally discriminated patients with and without GVHD. The area under the receiver operating characteristic curve distinguishing these two groups in the training set was 0.91 (95% confidence interval, 0.87 - 0.94) and 0.86 (95% confidence interval, 0.79 - 0.92) in the validation set. A model utilizing protein levels with 95% specificity for GVHD in the training set provided 94% specificity in the validation set. In patients with GVHD, Cox regression analysis revealed that the biomarker panel independently predicted survival independently of GVHD severity (p < 0.001, Table 1).

Table 1: Association of Maximum GVHD Grade and Biomarker Panel with Overall Survival

<table>
<thead>
<tr>
<th>Biomarker Panel</th>
<th>p value</th>
<th>Hazard Ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum GVHD Grade (12 vs 34)</td>
<td>2.35</td>
<td>&lt;0.001</td>
<td>2.11</td>
</tr>
<tr>
<td>95% confidence interval, 0.87 - 0.94</td>
<td>95% confidence interval, 0.79 - 0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biomarker Panel</td>
<td>2.46</td>
<td>&lt;0.001</td>
<td>2.43</td>
</tr>
</tbody>
</table>

HPLC and MALDI TOF MS analysis of novel antileishmanial compounds from Quassia amara

PALA1, CHAKRABORTY D1, BHATTACHARJEE S2, MAJUMDAR S2

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Background: Quassinoids are used in folk medicine for centuries and have antileukemic and antimarial activities. Aims: 1) To characterise and quantify quassinoids from Quassia amara bark tissue using HPLC and MALDI TOF. 2) To assess antileishmanial activity of purified quassinoid and neoquassinoid.

Results: The effects of two quassinoids on viability of promastigotes was 62µg & 98µg respectively. The ED50 value of quassin was 19.3µg/ml and that of neoquassin at 14min and 22.8µg/ml respectively. The effects of two quassinoids on viability of promastigotes were assessed by monitoring MTT metabolism after 96h cultured in the presence of 1-100ng/ml compound. Intracellular parasitic load of amastigotes/100 promastigotes was 62µg & 98µg respectively. The ED50 value of quassin was 19.3µg/ml and that of neoquassin at 14min and 22.8µg/ml respectively. The effects of two quassinoids on viability of promastigotes were assessed by monitoring MTT metabolism after 96h cultured in the presence of 1-100ng/ml compound.

Conclusions: A panel of four biomarkers can confirm the diagnosis of GVHD in patients at onset of clinical symptoms of GVHD and provide prognostic information independent of GVHD severity.

HPLC and MALDI TOF MS analysis of novel antileishmanial compounds from Quassia amara

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Background: Major limitations of using the PAMAM dendrimers for in vivo gene delivery application are low transfection efficiency, lack of target specificity, and limited transport into the nucleus of the target cells. Herein we report preparation and evaluation of ornithine-conjugated PAMAM dendrimers and their potential for cancer cell-specific gene delivery.

Methods: Ornithine-conjugated PAMAM dendrimers were prepared by Fmoc synthesis. A comparative gene transfection study between PAMAM dendrimers and the surface modified dendrimers was conducted in HEK 293T, GM7373, NCI H157G cell lines. Cytotoxicity of the dendrimers was tested in HEK 293T cells by MTT assay. Effect of excess of ornithine (100 µM) on transfection efficiency of the ornithine conjugated PAMAM dendrimers was investigated. A comparative transfection study in polyamine transport deficient (NCI H157R) and polyamine transport efficient (NCI H157G) cell lines was performed to confirm the role of the polyamine transporter system (PAT) in the dendrplex uptake.

Results: 1H NMR and MALDI-TOF spectral analysis showed that about 60 molecules of Ornithine-(PAMAM-ORN60) were coupled to a PAMAM dendrimer. Comparative study between cancer cell (NCI H157G) and HEK 293T cells showed higher transfection efficiency of PAMAM-ORN60 dendrimers in cancer cells than normal cells and the cells that were not exposed to dendrimer prepared at N/P of 10 were safe at concentrations below 50 µg/mL. Transfection efficiency significantly increased (χ) in increase in generation number and degree of ornithine conjugation. Transfection efficiency of the PAMAM-ORN60 dendrimers in presence of excess of ornithine while there was no effect on the parent PAMAM dendrimer. Transfection efficiency of the PAMAM-ORN60 was significantly low in NCI H157R (31.6±12.5%) as compared to NCI H157G cell line (63.1±8.6%).

Conclusions: Conjugation of ornithine significantly increased the transfection efficiency of PAMAM dendrimers. PAMAM-ORN60 dendrplex uptake was higher in cancer cells as compared to HEK 293T cells. Many tumor types have been shown to contain elevated polyamine levels and an activated PAT system. As the results demonstrated the role of PAT in the uptake of PAMAM-ORN60, they may serve as potential cancer cell-specific gene carriers.

### EHRILL II – 2nd World Conference on Magic Bullets

### Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich

### Nürnberg, October 3-5, 2008

### Surface-modified polyamidoamine (PAMAM) dendrimers for site-specific gene delivery

#### KUMAR A, YELLEPEDI VK, PALAKURTHI S

Department of Pharmaceutical Sciences, Irma Lerma Rangel College of Pharmacy, Texas A&M Health Science Center, Kingville, TX, USA

#### Background:

Major limitations of using the PAMAM dendrimers for in vivo gene delivery application are low transfection efficiency, lack of target specificity, and limited transport into the nucleus of the target cells. Herein we report preparation and evaluation of ornithine-conjugated PAMAM dendrimers and their potential for cancer cell-specific gene delivery.

#### Methods:

Ornithine-conjugated PAMAM dendrimers were prepared by Fmoc synthesis. A comparative gene transfection study between PAMAM dendrimers and the surface modified dendrimers was conducted in HEK 293T, GM7373, NCI H157G cell lines. Cytotoxicity of the dendrimers was tested in HEK 293T cells by MTT assay. Effect of excess of ornithine (100 µM) on transfection efficiency of the ornithine conjugated PAMAM dendrimers was investigated. A comparative transfection study in polyamine transport deficient (NCI H157R) and polyamine transport efficient (NCI H157G) cell lines was performed to confirm the role of the polyamine transporter system (PAT) in the dendrplex uptake.

#### Results:

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#### Conclusions:

Conjugation of ornithine significantly increased the transfection efficiency of PAMAM dendrimers. PAMAM-ORN60 dendrplex uptake was higher in cancer cells as compared to HEK 293T cells. Many tumor types have been shown to contain elevated polyamine levels and an activated PAT system. As the results demonstrated the role of PAT in the uptake of PAMAM-ORN60, they may serve as potential cancer cell-specific gene carriers.

### Targeted and Multifunctional Dendritic Polymers: Magic Bullets for Drug and Gene Delivery

#### PALEOS CM1, TISIOUVAS D2, SIDERATOU Z2, ITZIVELEKA LA2

1Dendrigen SA, Athens, Greece; 2NCISR ‘Demokritos’, Agia Paraskevi, Greece.

#### Background:

A significant number of molecules fail to be commercialized due to inability to be delivered to the appropriate tissues. Such bioactive molecules lack blood solubility, stability, tissue specificity and transport ability through cell membranes. For addressing these problems drug carriers based on dendritic polymers have been developed.

#### Methods:

Consecutive functionalization of these polymers leads to the preparation of multifunctional dendritic polymers which share the properties of drug delivery and controlled release. Thus, dendritic polymers have been developed bearing targeting ligands, which in addition to exhibiting the property of multivalency they are also complementary to cell receptors. Protective groups have also been introduced which prolong their circulation in biological fluids. Another crucial parameter for effective drug delivery systems is their transport through cell membranes. Finally, nanocarrier systems have been tailored in such a way that drug release is triggered by changes in the biological environment.

#### Results:

A schematic representation of a multifunctional dendrimer is shown below. Each group plays a specific role. Thus, specificity has been achieved by targeting ligands while transport through cell membranes has been achieved by molecular transporting moieties. Enhanced water solubility, decreased toxicity, biocompatibility, stability and protection has been achieved by functionalizing dendritic polymers with polyethylene glycol (PEG) chains. Finally, cationization of dendrimers induces the interaction with genetic material for the formation of complexes employed in gene therapy.

#### Conclusions:

Designed functionalization of dendritic polymers scaffolds results in the preparation of non-toxic nanocarriers of significant encapsulating capacity, specificity to certain biological cells and transport ability through their membranes.

### Potential Reproductive and Developmental Damage Induced by Metronidazole

#### PALERMO AM1, MERANI MS2, MUDRY MD2


#### Background:

Parasitic illnesses increase all over the world and metronidazole (MTZ) is the well-established antiprotozoal and antibacterial agent usually administered to children and adults at the reproductive age. Aim: To evaluate in vivo the effect of metronidazole on fertility or development.

#### Methods:

Reproductive effects in rats were evaluated by scoring 70 adult females (450 g/bw) treated with 250, 500 and 1000mg/kg/bw/day for 7 days. They were mated after treatments and sacrificed at 21 days of gestation. To study the stages of the spermatogenic cycle and spermatozoa morphology, 60 days old male mice received an MTZ dose of v/10 130 mg/kg/bw. Pachytene spermatocytes, spermatids stages and spermatozoa were analyzed. Developmental effects were studied by allowing wild-type female flies to lay eggs for 24 hr in media with MTZ at 0, 500, 1000, and 2000 µg/mL. Emerging flies (400 to1000 for each concentration) were examined. Control series (C) kept in standard conditions were always run.

#### Results:

In female rats MTZ did not affect pre-implantation deaths but increased the frequency of post-implantation deaths (C)<3%, T<14,9, 16, 20.5%) and of dominant lethals (C<3.9, T>12,0, 13, 17, 18%; P<0.05; Mann-Whitney U test) in exposed mice cellular composition and number of stages in the seminiferous tubules were not altered, but the spermatozoa morphology was severely affected (C=39,7±1,0, T=122,9±3,6; exposed mice cellular composition and number of stages in the seminiferous tubules were not altered, but the spermatozoa morphology was severely affected (C=39,7±1,0, T=122,9±3,6; Pr=0,0000; ANOVA Test). In flies, 1000 and 20000µg/mL MTZ-exposed series showed higher frequencies of total abnormalities (C=6% T=2,8, 3, 32%; P<0,05; t Test) 2) In female rats treatments affected post-implantation death and induced dominant lethals, but abnormal offspring was not increased, probably because conceptions with aberrations are eliminated during post-implantation period. 3) The alteration of spermatozoa morphology by MTZ could represent a potentially serious threat to the normal fertilization process. 3) The morphogenetic alterations induced in Drosophila could indicate a potential developmental toxicity of the drug.

#### Conclusions:

In summary, by applying a combination of structure based drug design and rigid physicochemical criteria, we were able to identify a new PDE5 inhibitor series with wide chemical scope. Utilisation of co-crystal structural information and the wide scope of the drug-like molecules enabled rapid transition into potent and selective leads. Focus on retention of inherently good physicochemistry throughout the project has enabled the identification of a range of potent and selective PDE5 inhibitors with good physicochemical properties. These leads led to a physicochemically optimised amide with the potential for once daily pharmacokinetics in
Targeting the vulnerability of cancer cell mitochondria to selectively induce apoptosis: Evaluating the efficacy of Pancratistatin as a non-toxic anti-cancer agent

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Department of Chemistry & Biochemistry, University of Windsor, Windsor, Ontario, Canada

Despite the aggressive research efforts to find selective anti-cancer chemotherapeutics, cancer remains unconquered. The major difficulty with the treatment of cancer is the non-specificity with which chemotherapy kills cells. Many current treatments, including radiotherapy, are damaging to both cancerous and non-cancerous cells. Non-specific damage causes harsh side effects and DNA mutations that increase the possibility of cells becoming cancerous. Pancratistatin is a natural compound that was isolated from the spider lily in 1992, by Petit et al., and has been shown to have anti-cancer properties. We have recently demonstrated that while Pancratistatin induces apoptosis (programmed cell suicide) in cancer cells it does not affect non-cancerous cells. We have also demonstrated the non-genotoxic nature of Pancratistatin; its ability to kill cancerous cells without targeting their DNA. We have investigated the specificity and biochemical mechanism of action of Pancratistatin; our results indicate that Pancratistatin specifically and effectively induces apoptosis in human prostate cancer, breast cancer, neuroblastoma, melanoma and leukemia cell lines. Interestingly, we have also demonstrated that Pancratistatin targets the mitochondria of cancer cells. Mitochondria from non-cancerous cells are not affected by this treatment, indicating that vulnerability to this compound is limited to cancer cell mitochondria. In vivo results with human colon and prostate cancer xenotransplants in immuno-compromised mice have indicated that Pancratistatin inhibits tumor growth and is well-tolerated at the effective dose. These results reveal a new opportunity for the development of chemotherapy that targets mitochondria of cancer cells and advance our knowledge of a novel mechanism of action for Pancratistatin.

The role of corticosteroids, male and female sex hormones in a complex with apolipoprotein A-I in the regulation of gene expression

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Apolipoprotein A-I (apoA-I) is a universal transport species for corticosteroids, female and male sex hormones. This protein performs the addressable delivery of hormones into cell nuclei. The initial unit of this mechanism is represented by receptor mediated endocytosis. In cell nucleus, a hormone – apoA-I complex interacts with gene promoters comprising (GCC), sequences and initiates their expression. However, the biological activity is inherent only in the steroid hormones that have a reduced Δ3,5-ketogroup in the A-ring. The molecular-biological methods, IR spectroscopy and small-angle X-ray scattering were used to reveal the mechanism of gene expression in a cell culture and eukaryotic DNA. The mechanism is related with competitive rupture of hydrogen bonds in complementary GC-pairs with participation of the CH-group in the third position of hormone A-ring. Further rupture of hydrogen bonds is caused by hydrophobic interaction between nitros base rings and hydrophobic regions of apoA-I. This creates conditions for deposition of RNA-polymerase onto DNA matrix and enhancement of gene expression. In a complex with apoA-I, such effect is observed with tetrahydroderivatives of steroid hormones, dehydroandrosterone, its sulfated form (dehydroandrosterone sulfate), pregnenolone, androstosterone, and other hormones.

In the organism, the A-ring Δ3,5-ketogroup of steroid hormones is reduced in resident macrophages that have a high 5α- and 5β-reductase activity. The macrophages entrap steroid hormones in HDL. The entrapment occurs via receptor mediated endocytosis. In secondary lysosomes of the cells, lipoproteins are disintegrated, which is accompanied by apoA-I release and reduction of Δ3,5-ketogroup of steroid hormones. Both compounds form the biologically active complex, which is secreted to intersitial space due to exocytosis. Further the complex is transferred to the somatic cell nuclei, where it participates in the enhancement of gene expression. The mechanism revealed in our work is non-specific. However, it becomes specific when occurring in the cells of target organs of the corresponding hormones, for example, in lymphocytes, reproductive organs, hormone-dependent neoplasms, etc.

Receptor Protein Tyrosine Phosphatase Beta/Zeta as a Possible New Target to Regulate Endothelial and Tumor Cell Migration

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Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, 26504 Patras, Greece

Background: Receptor protein tyrosine phosphatase β/ζ (RPTPβ/ζ) is a member of the receptor protein tyrosine phosphatases, characterized by the presence of an N-terminal carbonic anhydrase-like domain, a fibronectin type III domain and a serine, glycine-rich domain for chondroitin sulfate attachment in the extracellular region. It exists as four isoforms: long and short receptor forms and long and short secreted proteins containing the extracellular domains of the long and short receptor forms respectively. The cytoplasmic domain of RPTPβ/ζ contains two tandemly repeated phosphatase domains, of which only the membrane proximal is catalytically active, as well as a C-terminal PDZ-domain-binding motif, through which it interacts with other PDZ-domain-containing proteins. It is expressed in the nervous system and is involved in neuronal cell migration, differentiation, circuit formation and regulation of neuronal plasticity. RPTPβ/ζ is known to bind several extracellular matrix proteins and cell adhesion molecules and it is a receptor of the heparin-binding growth factors pleiotrophin and midkine. We have studied expression of RPTPβ/ζ in primary endothelial cells and several tumor cell lines, as well as its involvement in endothelial and tumor cell migration.

Methods: A combination of methods was used, including immunoprecipitation and western blot analysis, migration assays, siRNA, immunofluorescence and confocal microscopy.

Results: RPTPβ/ζ is expressed in endothelial, as well as diverse tumor cells and in all cases interacts with pleiotrophin and mediates its effect on cell migration. Interestingly, the effect of RPTPβ/ζ on cell migration can be modulated by its interaction with other cell surface molecules such as integrins. Moreover, RPTPβ/ζ seems to mediate cell migration induced by diverse factors, such as hydrogen peroxide, nitric oxide, aprotinin and vascular endothelial growth factor, an effect that may or may not be dependent on pleiotrophin.

Conclusions: RPTPβ/ζ seems to be an interesting target in order to limit endothelial and tumor cell migration.

This research project is co-financed by E.U.-European Social Fund (75%) and the Greek Ministry of Development-GSR (25%).
Neurosteroids in the treatment of neurodegeneration

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Background: The brain has the ability to convert cholesterol to steroids, otherwise known as neurosteroids. Various studies using either pharmacological tools modulating endogenous neurosteroid levels and behavior or correlation of neurosteroid levels with neuropathology suggested that neurosteroids could be targeted to treat disturbances in the nervous system. Alzheimer’s disease (AD) is a yet incurable degenerative neurological illness characterized by memory loss. We reported that 22R/hydroxycholesterol (22RHC), a steroidal intermediate formed during the synthesis of pregnenolone from cholesterol, is present at lower levels in the hippocampus and frontal cortex of AD patients than in corresponding tissue from age-matched controls. 22RHC was then found to protect against β-amyloid (Aβ) induced-neurotoxicity. Aβ, as well as the formation of Aβ oligomers and amyloid deposits has been linked to AD pathology.

Methods and Results: Because 22RHC is a rapidly metabolized intermediate in pregnenolone biosynthesis, the stable naturally occurring heterospirostenol, (22R,25(R)-20α-spirospiro-5-endo,3β-epoxy-hexanoate (caprospinol), was identified by in silico screening of commercial libraries as the lead substitute. Caprospinol was found to protect against Aβ1-40 induced-neurotoxicity in vitro. This steroid binds to Aβ1-40, inhibits the formation of neurotoxic amyloid oligomers, prevents Aβ1-40 from reaching neuronal mitochondria, and protects mitochondrial function against direct insults. To investigate the in vivo efficacy of this compound we used a rat model of AD, which recapitulates the histopathological and cognitive phenotype of AD. Caprospinol treatment improved learning and spatial memory, as assessed using Morris water maze tests. This recovery of cognitive function was accompanied by decreased hippocampal and Listeria spp. The antimicrobial factor in each case was identified as caprospinol. In parallel studies caprospinol administration resulted in an important accumulation of the compound at the forebrain demonstrating its ability to cross the blood-brain barrier. Caprospinol does not bind to any known steroid hormone receptors and is devoid of acute and 3-month toxicity in rodents.

Conclusions: These results position caprospinol as a promising drug candidate for AD treatment.

This work was funded by Samaritan Therapeutics (Quebec, Canada).

Isolation, purification, partial characterization, biochemical properties and stability of two novel antimicrobial peptides produced by Pedicoccus strains

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Background: Fermentation broths (in MRs medium) of Pedicoccus acidilactici NRRL B-5007 and P. pentosaceus SM-1, were found to exhibit strong antimicrobial activity against a wide range of food spoilage and food born pathogen bacteria, including Staphylococcus aureus and Listeria spp. The antimicrobial factor in each case was identified to be a peptide.

Methods: The broths were subjected to repeated tricine-SDS-polyacrylamide gel electrophoresis until the antimicrobial factor was identified by overlaying the gel with nutrient agar in which the indicator microorganism Micrococcus luteus was embedded. After 16 hours of incubation existing and developing new areas of inhibition were eluted directly into an aqueous solution of formic acid/water/2-propanol (1:3:2 v/v). Molecular mass determination by electrospray ionization mass spectrometric analysis (ESI-MS) revealed a 3,600 Da peptide in the case of P. acidilactici, while a 5,370 Da peptide in the case of P. pentosaceus.

Results: N-terminal sequencing determined by Edman degradation in both cases showed that the peptides have 19 amino acid residues and the consensus sequence of -YSGGV- near the N-terminal. The last characteristic classifies the peptides to the class Ila of bacteriocins, known as pediocins. The names of pediocin SA-1 from P. acidilactici and pediocin SM-1 from P. pentosaceus are assigned to the isolated peptides. The purified pediocins were examined for their sensitivity to proteolytic enzymes and for their stability to cold, heat and pH treatments (the parameters ranging widely). The most remarkable characteristics of pediocin SM-1 lost its activity in most cases. The mode of action of both pediocins was found to be bactericidal. Fermentation kinetics studies in bioreactors revealed the primary metabolite nature of the peptides.

Conclusions: Antimicrobial peptides produced by lactic acid bacteria are believed to hold the potential answer to the growing problem of resistance to conventional antibiotics. The fact that the two isolated pediocins are produced by well-known food grade bacteria, makes them important candidates for use as biopreservatives. The fact however, that the producer bacteria belong to the human intestinal lactic acid bacteria, makes them attractive targets for further research with the aim of the production of novel antimicrobial drugs or innovative drug delivery systems.

A Novel, Safe, and Effective Clinical Treatment to Eliminate Resistance in Ectoparasites

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Background: Head lice have been on the increase for over a decade, in large part due to the fact that they have become resistant to conventional treatment. Head lice (Pediculus capitis) feed every 4 to 6 hours on human blood by attaching to the scalp. The adult female louse usually lays eggs (nits) near the scalp and transfers them to a hair shaft. If they are not killed or removed, nits hatch in 7-10 days and propagate the infestation. We are studying a botanical derived product (BGC20-582 Louse Treatment Gel) that kills both lice and their eggs.

Methods: Pediculicidal activity (in vitro) was evaluated for BGC20-0582 in concentrations of 0% (vehicle), 2.5%, 7.5%, 10.0% and 12.5%. N IX®, an FDA approved 1% permethrin product, was used as an active control and water as a negative control. Five centimeters disks were cut from 100% cotton towels and placed in the bottom of 15 x 90 mm Petri dishes. Each formulation was measured at 0.7cc and spread across the bottom of the disk. Adults lice and nymphs, were distributed evenly between test samples and controls. Pediculicidal activity was observed over an exposure period of 5 minutes to 1 hour for each treatment. Ovicial activity (in vitro) was evaluated with the same concentrations and controls. Groups of 10 hairs, each with a viable egg, were cut to 2-cm long strands and grouped together at one end with small adhesive labels. Each group was immersed in a test product for 30 minutes, and then rinsed with purified water and air dried prior to being transferred into 1 dram sterile vials and labeled accordingly. The eggs were placed in an incubator for two weeks with 80 degree F. temperature and relative humidity of 70%-80%. Ovicial activity was determined by observing the number of eggs failing to hatch following the 14 day incubation period.

Results: Based on preclinical study results it is proposed that the required concentration and exposure time for BGC20-0582 to achieve maximal pediculicidal and ovicial activity in vitro is treatment with a concentration of 10% for a period of 30 minutes.

Conclusion: We have just concluded a 4 arm, randomized double blinded, placebo controlled dose ranging study on approximately 230 subjects between 6 months of age and 70 years. This population is known to have permethrin and pyrethrin resistant head lice. The results are being analyzed and will be available shortly.
EHLRICH II – 2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich
Nürnberg, October 3-5, 2008

Graft Cell Line-derived Neurotrophic Factor Family: Antimicrobial-Transcriptional Regulation, Neurite Outgrowth and Actin Polymerization in Mature Dorsal Root Ganglia Neurons

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Background: Neurotrophic factors are signaling molecules that regulate multiple aspects of the development of the nervous systems. The giall cell line-derived neurotrophic factor (GDNF) family includes artemin, persephin, and neurturin. GDNF was found to reverse the postranslational changes into nerve regeneration and neurotrophic pain treatment. Artemin expression is observed in the adult brain and spinal cord, suggesting that artemin may play a role in the post-developmental stage. We performed a microarray analysis and real-time PCR experiment to investigate the patterns of gene expression underlying the effect of artemin on the mature DRG.

Methods: An in vitro isolated DRG model was used to study the effects of artemin on the adult rat neuronal system and investigate differentially regulated genes. For the DRG neuron culture, the ganglia were trypsinized. Total RNA was extracted and reverse-transcribed to double-stranded cDNA using an oligo-dT primer. The cDNA microarray containing a set of 5088 rat cDNA was used. The cultures were stained for vesicle endocytosis with FM 1-43 and for F-actin with phalloidin–AlexaFluor 488.

Results: 285 genes were differentially transcribed by artemin after 3 hour of treatment. A series of genes involved in the regulation of actin dynamics, including coronin, Myr 5, Wiskott-Aldrich syndrome protein interacting protein, cofilin, drebrin and dynamin were down-regulated by artemin, suggesting a previously undefined role in the regulation of actin polymerization and synaptic vesicle movement. Artemin also down-regulated the expression of genes related to cell adhesion and matrix assembly, including laminin, plecanin, fyn, neurturin, and the neuronal-glia-CAM-related cell adhesion molecule, which is functionally relevant to neurite elongation in DRG neurons. Artemin resulted in increases in total neurite length and branching of the DRG neurons. Also artemin caused an increase of synaptic vesicle clustering. The inhibition of DNA methylation suppressed the artemin-dependent neurite growth.

Conclusions: (1) The mature DRG neurons showed some response to artemin, suggesting that they exhibited a developmental shift to the ligand as prenatal DRG neurons. (2) DNA methylation seems to provide a mechanism for artemin-dependent genetic regulation responsible for axonal growth.

Central glutamatergic dysfunction as an explanation of resistance and refractoriness in Obsessive-Compulsive Disorder

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About 50% of patients affected by Obsessive-compulsive Disorder (OCD) do not respond to pharmacological treatment with selective serotonin reuptake inhibitors (SSRI) and clomipramine, drugs considered the gold standard treatment for OCD. Resistant patients are those who participate in a trial with any first-line therapy and do not achieve a satisfactory response to the refractory patients, as those who do not respond appropriately to several treatments administered in an adequate manner. In patients who do not respond to the above treatments, therapeutic strategies include switching and augmentation. A number of studies have shown that switching strategies can determine an improvement of OCD symptoms in as much as 40% of refractory OCD patients. Other studies have demonstrated that clinical improvement can be obtained adding drugs acting on dopaminergic system (typical and atypical antipsychotic drugs). Recently, it has been shown that drugs acting on systems other than dopamine, in particular glutamatergic drugs can ameliorate clinical OCD symptoms. The glutamatergic system was included in the physiopathology of the OCD after the observation of an increase in the glutamate concentration in the caudate nucleus of children with OCD and normal glutamate levels after treatment with paroxetine. Increased glutamate levels in the LCR of OCD patients was also described. Drugs that modulate the glutamatergic system, as risuloze, d-cycloserine and memantine, were recently used for refractory OCD patients. Memantine blocks the N-methyl-d-aspartate (NMDA) receptor-associated ion channel, and acts as an uncompetitive, low-affinity, open-channel blocker that enters the receptor channel preferentially when it is excessively open. The NMDA receptor is normally a low-affinity, open-channel blocker that enters the receptor channel preferentially when it is excessively open. The NMDA receptor is normally activated by the binding of glutamate, the major excitatory neurotransmitter in the central nervous system. It is believed that increased influx of calcium ions from the excessive activation of this channel may lead to excitotoxic damage to neurons in the brain. Neurotoxicity due to central glutamatergic hyperactivity may explain refractoriness to serotoninergic agents. There is still a few data on the efficacy of the use of agents that affect the glutamatergic system of OCD patients however, the preliminary results seem to be promising.
Retinoic Acid Regulates the Expression of the Anti-Apoptotic Protein
PKCβII

PATENLA.1, COOPER DR.2

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Background: The human keratinocyte cell line, NT2 cells differentiate into NT2 neurons upon treatment with retinoic acid (RA). This is a widely accepted model to investigate the expression of genes involved in neurogenesis, neuronal differentiation and early development of nervous system. Protein kinase C (PKC) Δ plays an important role in the regulation of cell apoptosis. We have recently described a novel PKCδ isoform - PKCδIII that is expressed in human NT2 cells following RA treatment. PKCδIII and PKCδII are the alternatively spliced variants expressed in human cells.Expression of PKCδII peaks at day 1 of RA treatment and then declines by 7 days. RT-PCR analysis and sequencing data revealed that this isoform is generated via utilization of a downstream alternative 5′ splice site of exon 10 which results in an insertion of 93 bp in the caspase-3 recognition sequence within the V3 domain. We have shown that PKCδII is resistant to caspase-3 cleavage and that PKCδII regulates anti-apoptotic effects in these cells.

Methods: RNA isolation of NT2 cells treated with retinoic acid were performed using RNA Bee (Tel Test, Inc.). RT-PCR was performed using primers that detect both PKCδ isoforms as well as primers specific for PKCδII.

Results: We have identified the nuclear serine-arginine rich splicing factor SC35 (also called SRp30c) which expresses the promotion of PKCδII mRNA via utilization of the alternative 5′ splice site II on PKCδ exon 10. Western blot analysis demonstrates that the expression of SC35 increased with RA treatment concurrent with the increase in PKCδII expression. Overexpression of SC35 in NT2 cells promotes the expression of PKCδII. To further decipher the mechanism of alternative splice site selection we have designed and cloned a minigene which includes PKCδ exon 10 and its flanking introns in the pSP3L splicing vector. We showed that this minigene is responsive to retinoic acid. Further, over-expression of SC35 with PKCδ minigene promotes selection of 5′ splice site II transmission of cells with SC35 siRNA along with PKCδ minigene results in a decline of PKCδII expression.

Conclusions: (1) PKCδII plays a role in development of nervous system (2) RA regulates expression of PKCδII via SC35.

(-)-Bisabolol – A Specific Ergosterol Biosynthesis Inhibitor?

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Background: At the level of zymosterol in the ergosterol biosynthesis all subsequent intermediates are solely produced by fungi. The recognition of a specifically acting inhibitor within this part of ergosterol biosynthesis would lead to a new class of antifungal drugs. To obtain information on the existence of compounds having both, structural similarities to fungal ergosterol precursors plus anti-fungal properties, previous findings in literature were analyzed in view of the above-mentioned scenario.

Methods: A chemical substructure and MIC searchable computer database on antimicrobials (Amicbase) was used. In the data query antimicrobial compounds were searched, that have at least one of four structural similarities as they occur in section from zymosterol to ergosterol.

Results: A main chain of zymosterol was most frequently found. Addition of oxygen and limiting to Candida albicans resulted in 19 different compounds, whose MIC increased with molecular volume (r = 0.77). Like zymosterol this compound has a cyclohexane ring plus a second double bond in similar interatomic distance. This may point to an inhibition of zymosterol conversion into fucosterol by (-)-bisabolol, which would offer the opportunity to disturb specifically fungal biochemistry.

(1) Bisabolol from chamomile has antiinflammatory, wound-healing, antiinflamm., antispasmodic properties and inhibits fungi (3-100 ȝg/ml), gram- (+) (32-500), but less than gram- (-) bacteria. Due to its low toxicity (monkeys tolerated oral 15 ml/kg b.w.) and the lack of reports on allergic reactions in its cosmetic use, the compound was taken to treat fungal and bacterial infections. Own case reports will be given.

Conclusions: The antifungal mechanism of (-)-bisabolol is worthwhile for further investigation due to its safety and its unique pharmacological profile.

Differential roles of physiological and physicochemical parameters on local and variable bioavailability of Saquinavir- hurdles of effective drug treatment

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Background: Saquinavir (SQV), the first of the Human Immunodeficiency Virus (HIV) protease inhibitors to reach the market, remains one of the most widely prescribed agent which has markedly improved morbidity and mortality in HIV-infected patients. Inclusion of SQV in ‘highly active anti-retroviral therapy’ has substantially improved the clinical outcomes of AIDS patients. However, the complete therapeutic potential of this class of drugs is yet to be exploited due to number of limitations related to their poor and variable transport across important biological membranes, especially gastrointestinal tract. The pharmacokinetic profile of SQV being characterized by low and variable bioavailability is primarily attributed to metabolism by cytochrome P-450 3A4. Moreover, there is increasing understanding that membrane transporters (P-gp, MRP-2) contribute significantly to the biopharmaceutical characteristics of SQV and this entire class of drugs. However, the relative contributions of these eliminating organs to the first-pass effect and the significance of incomplete absorption of SQV have not been explored.

Methods: In this study, our objective was to determine the various factors governing the bioavailability of SQV. In order to understand the contributions of i) first-pass metabolism in gut and liver and ii) solubility and permeability of SQV across the lumen, towards its bioavailability, in-vivo, in-vitro and in-vivo studies results in rat model were compared. Though, the first-pass intestinal and hepatic metabolism has been shown to be a major determinant in the oral clearance of SQV, we found that pharmacokinetic characteristics may also have an important role in determining the oral absorption and disposition properties of this drug. To test of our knowledge, we are the first to report a double-peak phenomenon in plasma concentration-time profiles of SQV via oral administration. Although the double peaks in the plasma concentration-time profiles after p.o. doses could be attributed to a gradient expression of transporter proteins along the gastrointestinal tract, we hypothesize that the phenomenon is due to differential solubility profile of SQV across the intestine. In-vitro solubility data clearly indicate the pH dependent solubility profile of this drug. SQV has pKa values of 11.1 and 7.1, corresponding to the quinoline and cotiacetyloximeiminonitrogines, respectively. A base pKa of the drug just above pH 7.0, so the solubility of this drug would be expected to decrease significantly as the drug moves from duodenum (pH 8 to 9) to jejunum (about pH 7.8), and then decrease again in esophagus (about pH 5). The time of the second peak follows (4-6 hours) coincides with the expected variable intestinal delivery in the region, leading to a variable initial intestinal metabolism by法庭.

In order to assess solubility to molecular transporter characteristics, we speculated that an efflux (counter transport) mechanism might contribute to the low and variable bioavailability of SQV. Single pass in-vivo absorption method was employed for determination ofPartitionability (PDL) of SQV in various segments of rat intestine, via, duodenum, jejunum ileum and colon. The data revealed that the PDL of SQV through rat duodenum (2×10^-5 cm/s) is higher than jejenum (1.8×10^-5 cm/s) or ileum (2.1×10^-5 cm/s), which is in line with the higher extent of absorption of the drug in the duodenum. On the other hand, Fp, values of the drug in jejunum, ileum and colon were found almost similar. The results hence clearly suggest that SQV is a low permeable drug, apart from being poorly soluble at intestinal pH.

Conclusion: Because P-gp is found lower down the gut than is CYP3A, the exposure of SQV to P- gp is high because of its low solubility, and this could explain the very long period over which absorption is detected. Absorption, rather than elimination, controls the pharmacokinetics of SQV, and its very slowness is probably responsible for the low and variable kinetic profile of this drug. Therefore, in conclusion, the bioavailability of SQV is controlled by a combination of solubility in the gut lumen, p-glycoprotein mediated efflux in the gut, and first-pass intestinal and hepatic metabolism (CYP3A4). Given the differential and complex roles of physiological and physicochemical characteristics in SQV oral absorption, the optimization of AIDS treating regimes requires careful consideration in order to avoid therapy limiting drug-drug transporter and enzyme interactions.

Discovery and Use of the Magic Bullets in Human Taeniosis (Neciosamide, Praziqualant)

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Background: Taeniosis/cysticercosis is a serious public health problem in several countries of Latin America, Africa and Asia. Approximately 2 000 000 people carry a Taenia solium tapeworm. Globally, conservative estimates calculate 500 000 000 infections with T. solium. Many of these infections are in- deed asymptomatic and efforts to control taeniosis were treated with pumpkin seeds (cure rate of 88%, 226). Many of these drugs were unsaf e or poorly effective. Due to the high prevalence of taeniosis in the Americas the use of praziquantel in control of taeniosis is questioned, as this drug may be ineffective in taeniosis endemic areas.

Older taenicides: Several more or less toxic natural remedies, such as male fern extract, Kosso flowers, anea nuts, pomegranate have been used for centuries. In the early 20th century in Germany among 22 000 cases of taeniosis treated with male fern extract 18 patients died and 71 others have lost vision. Several synthetic drugs were tried as taenicides. The most important of those are thymol (1912), carbon tetrachloride (1931), hexylresorcinol (1932), mepacrine (1947), dichlorophen (1956), bithionol (1962), paromomycin (1967) and nitazoxanide (1996). During a clinical trial my patients with T. saginata taeniosis were treated with pumpkin seeds (cure rate 65% among 163 treated), atabrine (respectively 42%, 44), acarit (88%, 89) or praziquantel (94%). Therefore, these drugs are poorly tolerated, although some were rather effective.

Modern taenicides: The first magic bullet was - neciosamide, introduced in 1959. As a barely absorbed substance it is safe and well tolerated. However, it’s early original version (and still some generic products) has lost the efficacy during storage due to polymerization of its active particles. The efficacy of a single dose of neciosamide in human taeniosis is about 85%. Since 1972 niclosamide has been gradually replaced by the second magic bullet - praziquantel, being more effective and better tolerated, though praziquantel is more toxic (95%) and much cheaper (10 US cents for a dose). It has been used widely in the control of schistosomases. Due to autoinfection more than 10% of T. solium tapeworm carriers develop symptomatic taeniosis. Therefore, the use of praziquantel in control of taeniosis is questionable, as this drug may damage existing brain cysticerci and change asymptomatic neurocysticercosis into a symptomatic one. The problem is partly solved by reducing the dose of praziquantel in taeniosis to 5-10mg/kg b.w. in a single dose. Still some uncertainty exists whether it is worth to risk such a rare side effect at a mass-treatment in T. solium endemic areas.

Conclusions: (1) There is still a place for another magic bullet in taeniosis/cysticercosis. (2) Nitazoxanide, a broad spectrum antiparasitic drug, is waiting in a row.
Modifying cytoplasmic protein complexome involved in energy metabolism as a strategy of Escherichia coli cell efflux system

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Background: Recently, altered proteins or proteomes in response to antibiotic resistance are explored, but information required in the resistance is not available. Aims: 1) To establish a cytoplasmic protein complexome involved in antibiotic resistance (CRO) resistance by combined 2-D native/SDS PAGE and protein-protein interaction approaches; 2) To investigate functional characteristics of the complexome using genetic modified strains of the gene deletion of the proteins involved.

Methods: Cytoplasmic native/SDS PAGE and Co-IP, far-Western blotting or His-tag pull down assays were utilized for the cytoplasmic protein complexome of E. coli involved in CRO resistance. A modified 2-D native/SDS PAGE and protein-protein interaction approach was used to investigate CRO resistance. The complexome was investigated by minimum inhibitory concentrations (MIC) and survival capability assays of genetic modified strains of the genes deletion of the proteins.

Results: MaP, AtpD, PrB, LysT, MasE and CypK were found to be down-regulated, and Sulp, SucC and SucD were up-regulated in the CRO-resistant E. coli strain. They respectively belonged to seven protein complexes, MaP homodimer, AtpD homodimer, PrB-PrA, LysT homodimer, MasE-TatB, CypK-SodB and SucC-SodX. Most of them were involved in carbohydrate metabolism. MasE-TatB, SucC-SodX and LysT homodimer and CypK-SodB may play more important roles in the control of CRO resistance than other complexes identified. On the other hand, CRO-R and these mutants grew normally as the same as CRO-R-O in LB medium but most of them showed growth-combating in M9 medium with fructose, D-mannitol, malonte, glycerol or glucose. Importantly, up-regulated Sulp and SucD in 2-DE gels respectively played negative and positive role in regulation of TCA cycle and CRO resistance. All complexes but Sulp-SodX were contributed to down-regulated the classical carbohydrate pathways as a strategy against CRO.

Conclusions: Cytoplasmic protein complexome involved in CRO resistance of E. coli was achieved by 2-D native/SDS PAGE and Co-IP, far-Western blotting or His-tag pull down assays. Eight differential expressed proteins and three novel protein complexes, Trank-PrB and TatB-SodX were identified from the cytoplasmic fraction. Among these proteins, down-regulated of MaP and PrB, up-regulated of SucC-SodX, which involved in energy metabolism, were identified from the cytoplasmic fraction. These proteins, down-regulated of MaP and PrB, and up-regulated of SucC-SodX, which involved in energy metabolism, may play key roles related to resistance against the antitumor succinyl-CoA synthase and carbohydrate metabolism analyses using genetically modified strains with gene deletion of the proteins. These findings provide new insights into the mechanisms of CRO resistance, and these key proteins can be targets for development novel antibiotic compounds. In our knowledge, this is the first report on functionally altered complexome and energy metabolism modification to antibiotics-resistant bacteria. This work was supported by grants from National Basic Research Program of China (2006CB101807) and 963 project (2006AA023412).

Innovative Nanopharmaceutical Strategies for Neuronal Survival and Regeneration After Traumatic Brain Injury

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Background: Metallothionein (MT) is a multipurpose cytoprotective important for host defense responses, immunoregulation, and cellular survival. We have explored MT functions in the injured brain in vivo and tested the pharmaceutical effects MT. The aim is to develop treatment strategies for neurological patients.

Methods: This study applied genetically modified mice with MT deficiency or overexpression along wildtypes. The mice were subjected to a focal brain injury and in the following weeks, they received i.p. injection of exogenous MT protein or placebo. Animals were sacrificed at different time points and brains were removed for histopathology, molecular analyses and global gene profiling.

Results: In injured brain, MT inhibits inflammation by reducing macrophages, T lymphocytes and formation of proinflammatory interleukins, tumor necrosis factor-alpha, matrix metalloproteinases, reactive oxygen species and proapoptotic factors. MT enhances neuronal survival and brain repair processes including angiogenesis and neuroregeneration. The latter is due to activation and recruitment of resident neural stem cells and neuronal precursor cells. Both endogenous and exogenous MT had neuroprotective actions. Peripherally injected MT is well tolerated and is detected in the brain within 1 h after i.p. administration. This transport of MT from peripheral tissues into the brain is likely facilitated by blood-brain barrier leakage caused by the traumatic injury, which transiently disrupts integrity and permeability of the blood-brain barrier. The intracellular MT signaling is mediated in part by megalin, a multiligand endocytic receptor and is involved in lipoprotein receptor-related protein-1 (LRP). The intracellular signaling of MT involves kinases, phosphoproteins and other signaling mechanisms, G-protein coupled receptors, and zinc ion regulation, although the precise mechanisms downstream of MT remain to be fully established.

The data demonstrate the importance of MT for neuroprotective and regenerative responses following traumatic brain injury. MT is an antinflammatory modulation of energy production and function, which promotes brain tissue repair and functional recovery. MT may provide a novel pharmaceutical strategy against brain trauma.
Abstracts

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Adverse Reactions of Titanium-based Chemotherapeutic Agents on Male Reproductive Organs

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Background: Although the treatment efficacy of some titanium compounds as cytostatic agents on cancers is known, some side effects must be better estimated. Our previous studies have shown that titanocene dichloride, a metal-based antineoplastic complex, disrupted the blood-testis barrier in mice. Aims: 1) To compare the effects of two concentrations of titanocene dichloride on testis. 2) To estimate possible recovery of the lesions.

Methods: Male mice (2 months old) were subcutaneously administered with 0.12mg/g and 0.15mg/g/body weight of titanocene dichloride per se, and sacrificed after 72h. Other groups were injected similarly and kept for 35 days without any treatment. Controls were also considered. Animals were sacrificed for organs removal. Testes were fixed in Bouin’s solution and prepared for histology. Caudal epididymal fluid was collected for sperm quality analysis using standard procedures. Observations and micrographs were made using a light Nikon AFX-Dx microscope.

Results: A surviving rate of 100% was noted through the present study. However, histopathological studies have shown a considerable loss of germ cells after 72h, derangement of cellular organization within spermiogenesis of mice treated with the lowest dose. In comparison, the other group (0.15mg/body weight) showed more evidenced lesions. The sperm analysis from these groups of mice revealed a wide range of abnormalities in the concentration, motility, vitality, and morphology. After one cycle of spermatogenesis, a partial recovery of the lesions was detected in the testes and seminal fluid from the group administered with 0.12mg/g of titanocene dichloride. However, no reversible effects were detected in the highest dose group of animals.

Conclusions: 1) These results confirm that titanocene dichloride impairs at least spermiogenesis. 2) The persisting abnormalities observed after the recovery period suggest that this fact may compromise male fertility. Future studies must address the biochemical and molecular approaches of the blood-testis barrier, for the understanding of the pathophysiological mechanisms leading to the tests and epididymis alterations.

The differential dose-dependence for pro-angiogenic and cytotoxic effects of new anticancer plant agent within different nanocarrier formulations

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New drugs for cancer have been tested with a promising success. Molecular plant components were far ahead aimed into the angiogenesis and anti-angiogenesis pathways. Those molecules were able to destroy the microvasculature of cancer cells, what made scientists focus on the cancer cells themselves, not considering the destruction of the blood feeding system to eliminate the tumor. Another point was revealed as a difficulty was the poor capacity of these drugs to be administrated into an in vivo analyzing system, to prove other mechanisms of promoting the cancer cells death. The present paper deals with the construction of a nanocarrier system, in which a molecule of an anticancer plant agent was included. The effects of these new constructs were tested into the Chorioallantoic Membrane (CAM) in the chicken embryo model. A14 stimulates human umbilical vein endothelial cell (HUVEC) proliferation in the 1-10 micromolar range, and promoted cell death in the 1-10 micromolar range. It was also toxic to Murine melanoma B16F10 in 1-10 micromolar concentrations, even as proliferative effects were not observed. In vivo pro-angiogenic effect was observed in CAM, which presented increased number of blood vessel ramifications and capillary budding. The new vessels presented leaking and less organized than those from control membranes.

When molecular drug carriers were employed, the pro-angiogenic A14 effects could be observed at nanomolar concentrations, either in cell cultures or in the CAM model. Accordingly, treatment with A14 or its formulation dramatically reduced the number and size of the disseminated B16F10 murine melanoma tumors in the CAMs of live eggs. It is proposed that not only direct toxicity against B16F10 cell, but also the drug effects upon angiogenesis were relevant to both increasing tumor growth and inhibiting metastatic dissemination in the in vivo model of neoplasia.
High-dose Levofloxacin to Reduce Duration of Therapy and Slow Emergence of Resistance

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Background: The quinolone class of antibacterials was introduced more than 40 years ago with the accidental discovery of nalidixic acid as a by-product of the synthesis of the antimalarial compound, chloroquine. Nalidixic acid had a narrow spectrum of activity that targeted the most relevant pathogens; excellent pharmacokinetics including nearly complete absorption from the GI tract, broad tissue penetration, high serum concentrations and half-life suitable for once-daily dosing; levofloxacin was broadly adopted as the first true respiratory fluoroquinolone.

Methods: Extensive pharmacologic and clinical development of the quinolone class usually led to the discovery and development of the fluoroquinolone, levofloxacin.

Results: With a broad spectrum of activity that targeted the most relevant respiratory pathogens; excellent pharmacokinetics including nearly complete absorption from the GI tract, broad tissue penetration, high serum concentrations and half-life suitable for once-daily dosing; levofloxacin was broadly adopted as the first true respiratory fluoroquinolone.

Conclusions: Levofloxacin remains a potent and relevant antibacterial therapy today. Additional pharmacodynamic development of a high dose, short course treatment regimen of levofloxacin achieved the specific objectives of enhanced bacterial killing, reduced potential for emergence of bacterial killing, and has pioneered a paradigm shift towards short-course therapy of respiratory infections.

Tetracycline Biosynthesis: How to Overcome Nature’s Potential in Developing Novel Anti-infectives?

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Background: Tetracyclines (TCs) are large group of medicinally-important antibiotics with a common basic structure of four linearly fused six-membered rings. They are produced by several genera including Mycobacterium glutamicum, the oxytetracycline producer. Since, 1948, the first tetracyclines were developed - chlortetracycline, oxytetracycline, and tetracycline. They were amongst the first broad-spectrum antibiotics and their intensive use led to widespread microbial resistance. TCs are a very successful drug class - the tetracycline family is the largest family of antibiotics.

Methods: The unusual structure of the tetracycline biosynthetic pathway is based on a modular approach. The enzyme encoded by tcaR produces tetracycline synthase PKS, which is responsible for the production of tetracycline. PKS produces 10-12 tandem repeats of the tetracycline module, which is responsible for the production of tetracycline.

Results: Consistently with the chemical structure of the antibiotic, the cluster encodes genes for a typical minimal PKS (Ksg, Ksb and ACP), three genes involved in the cyclization/enantioselective process, methyltransferases, one aminodehydrogenase, oxygenases, a ketaotetradecase, an acyl-CoA ligase, a drug resistance transporter and a transcriptional regulator were identified. The application of bioinformatic engineering methods in combination with well-established semi-synthetic approaches will be presented.

Conclusions: The overall objective of this work was to clone, sequence and characterize a novel gene cluster encoding an unusual TC antibiotic. We report the identification of a novel tetracycline cluster which is suitable for further development as a novel antibiotic.

References:
PETKOVIĆ H, HUNTER IS, RASPOR P. Engineering of polykide biosyntheses: how close are we to the reality? Acta microbiol. pol. 500.
Prokinetic Effect Of Erythromycin: The Benefit Of A Common Side Effect

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Background: Erythromycin was firstly introduced in the clinical practice in 1950 as antibiotic. The prokinetic activity of erythromycin was discovered in the 1980's as an adverse effect of the drug. Since then erythromycin has been used in patients with chronic functional pseudo-obstruction, gastro-oesophageal reflux post-operative intestinal dysmotility, gastroparesis secondary to diabetes, hyperglycaemia and scleroderma and after surgical vagotomy. Its use as a prokinetic agent has also been extended to preterm infants with gastrointestinal dysmotility and feeding intolerance and more recently within the context of critically ill patients. It is effective as a prokinetic drug on gastroparesis related to acute pain-related stress and when given prior to elective surgery has been shown to improve gastric motility. However, the aim of the present study was to investigate whether Erythromycin accelerates the delayed gastric emptying of solids and hypertonic liquids induced by hyperglycemic conditions.

Methods: Twelve healthy subjects ate standard radiolabelled solid and hypertonic liquid meals. Gastric emptying was measured by scintigraphy during normoglycemia (5-8.9 mmol/L glucose) and hyperglycemia induced by intravenous glucose (16-19 mmol/L glucose) after administration of placebo or 200 mg of erythromycin intravenously. Emptying was measured randomly on 4 different days.

Results: Administration of erythromycin during normoglycemia or induced hyperglycemia compared with placebo, accelerated the gastric emptying of the solid meal while gastric emptying of the hypertonic liquid was reduced. Erythromycin versus placebo significantly reduced the lag-phase duration. The lag-phase duration was significantly increased (7.5 +/- 5.5 min and 7.2 +/- 4.5 min vs 10.5 +/- 3.4 min, and 3.5 +/- 2.5 min, respectively, P < 0.0001) as were the overall T1/2 (gastric emptying time of the half meal) (62.5 +/- 13 min and 24.5 +/- 5.5 min vs 42 +/- 10.5 min, and 16 +/- 6 min, respectively, P < 0.0001) and the percentage of meal retained in the stomach at 60 and 100 min postprandially (P < 0.0001).

Conclusions: Erythromycin-induced effect on gastric emptying of solids and hypertonic liquids is related to the plasma glucose level. Erythromycin accelerates gastric emptying rate of both solids and hypertonic liquids in both conditions, either in normoglycemia or hyperglycemia.

Magnetic bullet or magic diet: The need for further understanding in sport performance enhancements

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Background: Translational nutrition embodies the use of dietary components to effect a therapeutic end point commonly associated with medicinal intervention. For example, recent advances have been made in the study of dietary chelators for treating iron-related diseases, iron overload, plaques and by formation of anti-oxidant enzyme mimetics. Further work has posited dietary components could act as prebiotics and probiotics in the form of gene expression enhancers to enhance key protective enzymes such as superoxide dismutase. However, many scientific studies have focused on individual components of diet (e.g. anti-oxidants) with little or scant regard for the entire profile of a foodstuff. In this era of multiple advances in integrative translation nutrition, further emphasis on the informed decision making regarding nutrition is warranted. The present study is to determine decision making rationale versus practice for functional nutrition use in elite athletes.

Methods: The 'UK Sport 2005 Drug Free Survey' data (n = 874 adult and n = 403 youth elite athletes) were re-analysed using association [chi-square] and 'strength of association' tests [phi] to show the proportion of informed choices and to unveil incongruences between self-reported supplement use and the underlying motives.

Results: Participants reported supplement use for performance enhancing and health maintenance reasons. Of the 30 possible associations between supplements and reasons, 11 were predictable in the first and 10 in the latter category from literature precedents. In the adult athlete population, only 8/11 were evidenced and these were not strong (phi < .7). The best associations were for the ability to train longer and maintaining strength with creatine and whey protein. Association coefficients were found in 8/10 test pairs however only weak associations exist. Of these, 4 were associated with avoidance of sickness (iron, multivitamin, vitamin C and Echinacea). Similar results were found among young elite athletes, where supplements were taken for performance enhancing reasons. No agreement was observed between athletes' rationale and behaviour except for creatine.

Conclusions: These results suggest that a lack of understanding exists in supplement use. There is an urgent need to provide accurate information which will help athletes make informed choices about the use of supplements.

Combinatorial Nanobiotechnology – A Paradigm Shift in Chemistry and Material Science

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Background: The more than 20-year evolution of phage display has dramatically affected the potential of this technique amid other bioengineering methods. The marriage of combinatorial chemistry and biological selection has been very powerful changing the methodology of biochemical research by allowing selection simultaneously among billions of genetic species in one test tube. Genetically driven phage nanobiotechnology has allowed development of libraries of diverse nanostructures expressed on the phage surface providing a rich resource of diagnostic, detection and pharmaceutical probes.

Methods: Phage engineering, which is based on natural mechanisms of selection, amplification and self-assembly, allows directed nano-fabrication of bioselective materials with possible applications in gene/drug-delivery, biosensors, nanoelectronics, biosorbents, and other areas of medicine, technology, and environmental monitoring. In particular, landscape phage expressing tumor-specific peptides fused to all copies of the major coat protein pVIII can be converted easily into gene-encapsulating particles or drug-loaded vesicles that acquire the ability to recognize the same receptors, cells, tissues and organs that have been used for selection of the precisely targeted phage. The fusion major coat protein constitutes 98% of the total protein mass of the virion – a purity hardly attainable in normal synthetic and bioengineering procedures. As a normal intestinal parasite, phage and its components are not toxic and have already been tested for safety in preclinical and clinical trials. All these unique characteristics of phage commend it very well as a very promising nanomaterial for a variety of medical and technical applications. To illustrate the concept, the author will present the data obtained in his research group and collaborate research.

Results: This presentation focuses on the progress made in the development of these new nanomaterials and discusses the prospects of using phage as a bioselectable molecular recognition interface in medical and technical devices based on the experience of the author in this area.

Conclusions: Phage display evolved into a discipline of material science presenting phage not only as an instrument for peptide and antibody discovery, but also as a prospective nanomaterial that can be easily tailored using routine genetic engineering manipulations. This merge of phage display technologies with nanobiotechnology during the last several years is very promising and has already shown its vitality and productivity contributing vigorously to different areas of medicine and technology, such as medical diagnostics and monitoring, molecular imaging, targeted drug and gene delivery, vaccine development, as well as bone and tissue repair.

MRI monitoring of blood brain barrier alterations in inflammatory lesions of Multiple Sclerosis: Tools to evaluate disease activity, efficacy of treatments and to develop new therapeutic strategies

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Since 1999 we have pioneered a new approach of cellular and molecular imaging in vivo application to MS. In an experimental animal model of MS we have monitored the infiltration of the CNS by inflammatory blood cells (monocytes, T cells) labelled with iron nanoparticles as contrast agent in comparison to G adolinium enhanced MRI monitoring of blood brain barrier alterations in inflammatory lesions of the CNS. Since 1999 we have pioneered a new approach of cellular and molecular imaging in vivo application to MS. In an experimental animal model of MS we have monitored the differentiation of gene expression enhancers and their effect on gene/drug-delivery, biosensors, nanoelectronics, biosorbents, and other areas of medicine, technology, and environmental monitoring. In particular, landscape phage expressing tumor-specific peptides fused to all copies of the major coat protein pVIII can be converted easily into gene-encapsulating particles or drug-loaded vesicles that acquire the ability to recognize the same receptors, cells, tissues and organs that have been used for selection of the precisely targeted phage. The fusion major coat protein constitutes 98% of the total protein mass of the virion – a purity hardly attainable in normal synthetic and bioengineering procedures. As a normal intestinal parasite, phage and its components are not toxic and have already been tested for safety in preclinical and clinical trials. All these unique characteristics of phage commend it very well as a very promising nanomaterial for a variety of medical and technical applications. To illustrate the concept, the author will present the data obtained in his research group and collaborate research.

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Ongoing experimental work aims to identify molecular alterations at the BBB that occur at early stages in EAE rats, by phage display screening in CNS characterized by macrophage infiltrates with MRI. We further develop a strategy of using the MRI cell marker and peptide-based binding approach to inflammatory lesions sites for transport of therapeutic compounds into the lesion sites and their local liberation under the control of MRI.

All abstracts are listed in alphabetical order of the presenting author.
Metals as Endocrine Disruptors in Women's Reproduction: Assessment of Effect and Mechanism of Action in Different Steroidogenic Cells

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Background: The polluted environment contains a mixture of reproductive toxicants that includes metals and metalloids. The emerging evidence exists that cadmium, lead, arsenic, mercury and the others can act as endocrine disrupting chemicals in mammals. They can alter ovarian and/or placental steroidogenesis and thus affect ovarian cyclicity, the maintenance of pregnancy, and embryo/foetal development.

Methods: We conducted complementary research on cadmium-related steroid disruption using different experimental paradigms. Human placenta was used for ex vivo (epidemiological) and in vitro studies of cadmium effect(s) on placental progesterone production. In experiments on laboratory rats in vivo and in vitro and in the stable porcine granulosa cell line JC-410, steroidogenesis was assessed in the stable porcine granulosa cell line JC-410, steroidogenesis was assessed in the stable porcine granulosa cell line JC-410, steroidogenesis was assessed in the stable porcine granulosa cell line JC-410, steroidogenesis was assessed in the stable porcine granulosa cell line JC-410, steroidogenesis was assessed in the stable porcine granulosa cell line JC-410, steroidogenesis was assessed in the stable porcine granulosa cell line JC-410.

Results: In either human or rodent placenta and in ovary, increased cadmium concentrations in steroidogenic tissue were accompanied by decreased progesterone production. Direct cadmium effects on specific components of the steroidogenic pathway were found. This includes two sites of action: the low-density lipoprotein-receptor and P450 side chain cleavage enzyme. In cultured porcine granulosa cells, cadmium stimulated ovarian progesterone synthesis through a mechanism involving activation of P450 side chain cleavage gene expression.

Conclusions: 1) Cadmium has the potential to disrupt steroidogenesis in human placenta. 2) Cadmium may display paradoxical dual effects in the ovary: depending on the exposure level, it may either inhibit or enhance/mimic the biosynthesis of progesterone and oestradiol, and act as xenoestrogens (metalloestrogens). 3) Sites of cadmium direct effects on specific components of the steroid biosynthetic pathway are multifaceted and it is possible that cadmium's effects are “tissue-specific” in different steroidogenic cells.

Cardiiological medicines: allowed and prohibited pharmacological helping for athletes

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Background: Cardiological medicines are recommended athletes with medical indications by doctors working in a Center of Sport Medicine in Warsaw, Poland. There is almost two hundred consultations a year. The Therapeutic Use Exemption (TUE) allows athletes to use substances and methods banned by World Anti-Doping Agency (WADA). Diuretics are prohibited for sportsmen at all time (in and out of competitions). B-blockers are not allowed only in a particular sports (for example: ski jumps, snowboard or aerial and car sports). On the other hand l-blockers or cardioprotective substances like a trimetazidine are not yet present on the WADA list of prohibited substances.

Methods: Department of Anti-Doping Research of Institute of Sport in Warsaw, Poland (WADA accreditation since 2004) had been analyzed 6210 urine samples of Polish athletes since 2005 to 2007. This material has been tested retrospectively in direction of finding diuretics, b-blockers and their metabolites, l-blockers and cardioprotective substance - trimetazidine.

Results: - diuretics were found in 7 samples - b-blocker – only one case - l-blocker - trimetazidine was present in 6 athlete’s samples (all cycling riders in competition)

Conclusions: 1. The use of diuretics and b-blockers by athletes is the occasional phenomenon in Poland compared to the use of other banned substances such as anabolic steroids. 2. Trimetazidine and bufomedil are used as allowed pharmacological helping for athletes in Poland. 3. There is no objective evidence of clinical trials of trimetazidine and bufomedil use in improving the physical capacity at the athletes. 4. The use of trimetazidine and bufomedil in enhancing the capacity of physical athletes, wakes up ethical doubts but is not prohibited.
Cognitive dysfunction, hallucination and delusion are typical disorders that are diagnosed in schizophrenic patients and that can be induced in humans following the administration of a single non-anesthetic dose of ketamine, a non-competitive NMDAR antagonist. What is the impact of a similar single injection of ketamine in the EEG of rodents? And what is the direct effect of ketamine in cortical networks?

In awake adult rats, a single subcutaneous injection of ketamine (+5 mg/kg) induces persistent aberrant gamma frequency (30-80Hz) oscillations (increased power and intrinsic frequency) in the frontoparietal cortex and a concomitant ataxic behaviour. EEG recordings performed in deeply anesthetized rats demonstrated that these pathophysiological gamma oscillations are not caused by abnormal motor activity. Neither are they dependent on conscious sensorimotor processing. Local application of ketamine produces a cortical focus of persistent aberrant gamma oscillations, which progressively spread to adjacent networks. Furthermore, ketamine significantly increases the synchrony of basal gamma oscillations between two highly and not between two weakly interconnected structures. Juxtaocular recordings combined with EEG recordings have revealed that aberrant gamma oscillations are associated with a significant increase in the firing rate in the majority of glutamatergic corticofugal and thalamocortical neurons.

Our findings suggest that ketamine-induced persistent gamma hypersynchrony is an aberrant network noise that might set out of control the spatiotemporal patterning of inputs in cortical-related networks, which would impair top-down processing. A persistent decrease or annihilation of the signal/noise ratio of the cognition-related transient gamma synchrony might cause cognitive dysfunction, acute psychosis and exacerbate schizophrenia symptoms. Therefore, this persistent aberrant gamma noise may be a potential neurophysiological marker of psychoses.

In ULP support.

Anabolic Androgenic Steroids (AAS) Elicit Aggression by Selectively Decreasing Neurosteroid Biosynthesis in Corticolic Glutamatergic Neurons

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Background: AAS abuse is a serious problem among adolescents, elderly subjects, and military personnel. AAS increases sex drive and mental acuity but have remarkable physical and behavioral side effects, including irritability, aggression, and depression. The molecular mechanisms and neuronal circuitry underlying these symptoms are largely unknown. Protracted testosterone propionate (TP) administration in mice induces aggression, anxiety and a decrease in hippocampal 5α-reductase (5α-RI) and 3α-hydroxysteroid dehydrogenase (3α-HSD) activity in the hippocampus, cortex and thalamus, and results in decreased plasma levels of 5α-DHT. TP reduces the expression of 5α-RI and 3α-HSD colocalize in cortical, hippocampal and olfactory bulb glutamatergic neurons and in output neurons of the basolateral amygdala (BLA), thalamus, and striatum.

Methods: To study the alterations of selective neuronal circuitry in AAS abuse, we established a mouse model of AAS-induced aggression. Using GC-MS, we studied whether TP, in doses that induce aggression, selectively downregulates 5α-RI in a structure-specific manner. Using immunohistochemistry, quantitative nested RT-PCR, and Western blot, we studied whether TP decreases corticolic 5α-RI levels by downregulation of 5α-RI mRNA and protein expression. We used local micropunctations of the selective brain stereodendritic stimulant (SSBS), S-norfloxacin (S-NFLX), to upregulate 5α-RI and decrease aggression.

Results: Treatment of mice with TP reduces 5α-RI expression in selected glutamatergic pyramidal neurons of the cortex, hippocampal CA3, and BLA and in granular cells of the dentate gyrus, which results in a decrease of 5α-RI content. In contrast, 5α-RI mRNA expression fails to change in the striatum medium spiny neurons and reticular thalamic nucleus neurons, which are GABAergic. A bilateral micropuncture of S-NFLX in the BLA decreased TP-induced aggression by upregulation of 5α-RI levels.

Conclusions: TP-induced aggression is associated with an impairment of 5α-RI biosynthesis in specific corticolic glutamatergic neurons. These results may help in the design of therapeutics to limit the adverse effects of AAS abuse.

IL-12 receptor expression and function on human lung adenocarcinoma: identification of a new potential therapeutic target

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Background: Non small cell lung cancer (NSCLC) is a leading cause of cancer death. While a lack expression of the β2 chain of the interleukin-12 receptor (IL-12Rβ2 KO mice) develops spontaneously lung adenocarcinomas or bronchiolaralveolar carcinomas. Here we have investigated i) IL-12 inhibits directly the growth of human lung adenocarcinoma; ii) IL-12 induces apoptosis, iii) β2 on primary lung adenocarcinoma; iv) the direct activity of IL-12 on NSCLC cells and the mechanisms involved.

Methods: Lung adenocarcinomas tissues obtained at diagnosis from seventy untreated patients were studied for IL-12Rβ2 expression by immunohistochemistry. Stage I lung adenocarcinomas showed significantly (P<0.012) higher frequency of IL-12Rβ2 compared to stage III/II tumors. Calu-1 NSCLC cells were next transfected with IL-12Rβ2 containing plasmid (Calu6β2), while NBEBC cells were expanded from lung samples of non-neoplastic origin. In vitro IL-12 activity on Calu6β2 or NBEBC was investigated by flow cytometry, ELISA and chromoantigenic membrane assay. Severe combined immune deficiency (SCID)/ non obese diabetic (NOD) mice were inoculated with Caluβ2 cells subcutaneously or orthotopically and treated with human recombinant (hr) IL-12. Explanted tumors were studied by polymerase chain reaction (PCR) array and immunohistochemistry.

Results: IL-12 treatment of Calu6β2 cells inhibited IL-6 production and angiogenesis in vitro. Tumors formed by Caluβ2 cells in SCID/NOD mice were significantly smaller following hrIL-12 vs PBS treatment due to inhibition of angiogenesis. NSCBE expressed functional IL-12 and IL-12 dampened the release of cytokines involved in tumor progression.

Conclusions: 1) IL-12 inhibits directly the growth of human lung adenocarcinoma and targets the adjacent NBEBC, 2) IL-12Rβ2 on primary lung adenocarcinoma may represent a new therapeutic target, and 3) clinical trials investigating hrIL-12 activity in lung adenocarcinoma patients appear feasible.
Silencing Cell-Cell Communication: The New Bullets in Anti-Infectives

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Background: At least two lines of communication occur in the host: bacterial cell-cell communication (quorum sensing) and cross-kingdom communication, i.e., host hormones functioning as molecular signal mimics. Both forms of chemical communication can affect phenotypic expression of virulence factors and antibiotic activity. Currently, focus is on design of quorum sensing autinducer analogs. However, cross-kingdom communication presents a particularly intriguing line of communication, with evolutionary origins of some host hormones and toxins as well. To fulfill their role as inter-kingdom communication molecules, the effect of insulin and dehydroepiandrosterone (DHEA) on phenotypic expression was assessed.

Methods: Staphylococcus aureus 29523 grown with DHEA (0.1, 0.5, 1.0, 5.0 M) was tested for susceptibility to Triton X-100, lysozyme, β-defensin and vancomycin. β-Insulin’s (Humulin) role as a microbial insulin mimic was tested in Escherichia coli K12 by measuring the effect of insulin (2-400 μU/ml) on E. coli adherence, biofilm production and chemotaxis, using standard methodology.

Results: S. aureus exposure to DHEA resulted in increased carotenoid levels which correlated with increased resistance to Triton-X100, lysozyme, β-defensin and vancomycin. Exposure of E. coli to β-insulin affects E. coli behavior. Insulin alone is a chemo-repellent. However, with glucose insulin enhances glucose chemotraction, adherence (glass, plastic and latex) and biofilm formation as compared to sugar alone.

Conclusion: 1) DHEA enhances carotenoid synthesis and resistance to cell-wall active agents. 2) β-insulin in the absence of glucose disperses populations in nutritionally impoverished environments; however, with glucose present insulin enhances population density through biofilm formation. It is possible that in some cases, inter-kingdom signaling molecules may provide an alternative signaling pathway that will need consideration along with more traditional quorum autoducers if analogs of this class of compounds will have broad utility as anti-infectives.

Treating fungal infections with CYP53A15 inhibitors

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Our in vitro and in vivo studies suggest that targeting CYP53A15 could help treat fungal infections. In vitro, four phenolic compounds (isoeugenol, eugenol, vanillin and thymol) that play a role in plant resistance to fungal infection inhibited CYP53A15. Inhibition of CYP53A15 leads to increased intracellular levels of benzoic acid, which impedes fungal growth. In vivo, three of these compounds inhibited Cochliobolus lunatus growth. Next steps include solving the X-ray crystal structure of the CYP53A15 active site.

Status and antibiotic sensitivity profile of Salmonella enterica during 2001-2007 at Tribhuvan University Teaching Hospital - a tertiary health care centre in Nepal

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Background: Enteric fever is a common problem in Nepal. In view to determine the trends of Salmonella causing bacteremia/sepsisemia and to determine the antimicrobial susceptibility profile of these organisms, a retrospective study was carried out during 2001 to 2007 at Tribhuvan University Teaching Hospital, a tertiary care health centre in Nepal.

Methods: During 2001-2007, 41408 blood culture samples from the patients clinically suspected of enteric fever were received at Department of Microbiology, Tribhuvan University Teaching Hospital, Katmandu, Nepal. These samples were cultured and isolates were identified then subjected for antibiotic sensitivity testing as described by American Society for Microbiology (ASM).

Results: Approximately 13% blood culture samples showed growth positive. Of which about 10% were Salmonella enterica serotype Typhi and Paratyph-A. Three percentages of isolates included Shigella boydii, E.coli, K. pneumoniae, Pseudomonas spp, V. cholerae, Co. jejuni, Citrobacter spp, Enterobacter spp and Streptococcus faecalis. Among 4031 Salmonella isolates, 6.5% were resistant to at least two groups of antibiotics. Almost equal percentage of both salmsonella enterica serotype Typhi and Paratyph-A isolates were found to be multi drug resistant (MDR). Many of the multi drug resistance Salmonelle were resistant to amnillin, ciprofloxacin, co-trimoxazole and chloramphenicol.

Conclusion: There is increasing trends of Multi drug resistant Salmonelle over the years, therefore it is felt that the cause of resistance should be investigated.

The Use of Regulatory RNA Molecules as a Novel Treatment Strategy for Cardiac Diseases - RNA as a Magic Bullet?

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The current status and challenges of RNA interference (RNAi) and microRNA modulation strategies for the treatment of myocardial disorders are discussed and related to the classical gene therapeutic approaches of the past decade. We summarize the key issues of current vector technologies which determine if they may be suitable for clinical translation of experimental RNAi or microRNA therapeutic protocols. We then present and discuss examples dealing with the potential of cardiac RNAi therapy. First, an approach to block a key early step in the pathogenesis of a virus-induced cardiomypathy by RNAi targeting of a cellular receptor for cardiomypathic viruses. Second, an approach to improve cardiac function by RNAi targeting of late pathway of heart failure pathways common to myocardial disorders of multiple etiologies. This strategy is directed at myocardial Ca2+ homeostasis which is disturbed in heart failure due to coronary heart disease, heart valve dysfunction, cardiac inflammation, or genetic defects. Whereas the first type of strategies (directed at early pathogenesis) need to be tailor-made for each different type of pathomechanism, the second type (targeting late common pathways) has a much broader range of application. This advantage of the second type of approaches is of key importance since enormous efforts need to be undertaken before any regulatory RNA therapy enters the stage of possible clinical translation. If then the number of patients eligible for this protocol is large, the actual transformation of the experimental therapy into a new therapeutic option of clinical importance is far more likely to occur.

Modern quantum chemical descriptors for QSAR/QSPR

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Background: The current availability of cheap computing power enables solving the Schrödinger equation for drug-sized molecules. Modern ab initio methods yield electronic properties of geometry-optimized molecules. A new theory called Quantum Chemical Topology (QCT) (based on “Molecules”) provides descriptors for QSAR/QSPR. These modern and more realistic descriptors capture electronic effects and replace the Hammett constants.

Methods: QCT locates Bond Critical Points (BCPs) in 3D space. Properties such as the electron density or its Laplacian describe the bonds. A molecule is compactly represented in an abstract space of BCP properties. Partial Least Squares and various machine learning techniques can map the QCT descriptors to a wide variety of activities of medicinal, ecologival (toxicological) interest. Cross-validation and randomisation testing protect against chance correlation. True external validation features in our recent studies. Our method can localize a part in the molecule where the chemical change associated with the observed activity actually happens.

Results: In the case of the antinmucour activity of phenylbutenones we confirmed the hypothesis that these compounds act via a Michael addition. The context of mutagenic activity we determined a preferred mechanistic pathway for the initial hydroxylation of dimethyl hyotrotransaminases, a hitherto ambiguous issue. We studied seven datasets: (1) pIC50 of substituted imidazolines and (2) imidazoles, (3) the ability of indole derivatives to displace [3H]fluorotiazepam from binding to bovine cortical membranes, (4) the inhibition of β-glucuronidation constants for benzimidazoles, (5) the interaction constants for amidox and the enzyme liver alcohol dehydrogenase, (6) the natriuretic activity of sulfonamido carbamic antihydrase inhibitors and (7) the toxicity of benzylic alcohols. Hepatotoxicity and nitroaromatic toxicity were also investigated.

Conclusions: Electronic effects can be captured by a novel class of QCT descriptors that come directly from ab initio wavefunctions. Active sites in molecules are highlighted and robust predictions made for diverse activities.


All abstracts are listed in alphabetical order of the presenting author.
Exploring Leukotriene B4 (LTB4) as a periodontal biomarker: A time to focus.

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Background: Leukotriene B4 (LTB4) is a membrane-derived lipid mediator formed from arachidonic acid. LTB4 is among the most potent stimulants of polymorphonuclear leukocytes (PMNs) and, thus, participates in tissue injury by recruiting PMNs in a pathophysiologic scenario of periodontal and other systemic diseases. The aim of the present study was to assess the relationship between LTB4, clinical parameters and components of gingival crevicular fluid (GCF) from inflamed gingiva and periodontitis sites before and after the treatment of periodontitis.

Methods: Study subjects were divided into three groups with 20 subjects in each group: healthy (group 1), gingivitis (group 2), and chronic periodontitis (group 3). All the subjects were treated with subgingival scaling and root planning (SRP). GCF samples collected from each patient were quantified for LTB4, using an enzymatic immunometric assay. In addition, the correlation between LTB4 levels and clinical parameters was analyzed in each group.

Results: The highest mean LTB4 concentration in GCF was observed in group 3 (185.2 pmol/mg/mL), and the lowest was observed in group 1 (38.9 pmol/mg/mL). LTB4 level in group 3 decreased to 78.35 pmol/mg after treatment (group 4). Further, GCF LTB4 levels in all groups showed a statistically significant positive correlation with PDI and CAL (P < 0.005).

Conclusion: The substantial increase in GCF LTB4 concentrations with the severity of periodontal disease and a concomitant decrease in its level following SRP in subjects with periodontitis suggest a possible role for LTB4 as a biomarker in the progression of periodontal disease and aid in our understanding of the pathogenesis and the appropriate treatment of periodontal diseases by utilization of LTB4 inhibitors and antagonists to LTB4 receptor as ‘magic bullet’ that would bypass the need for professional and self-administered oral prophylaxis protocols.

Sperm immobilization factor: potential candidate for fertility control and antibacterial targeting bacterial motility

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Unplanned pregnancies present a great risk to the reproductive health of women. Therefore, female-controlled vaginal products directed towards contraception are needed urgently. In the present study efforts have been made to evaluate the contraceptive potential of sperm immobilization factor (SIF) isolated from Staphylococcus aureus isolated from an infertile woman. The results showed that sperm motility was completely inhibited by SIF along with complete loss of viability. The effect on the sperm motility was found to be dose and time dependent. The minimum effective concentration of SIF required for complete immobilization of sperm was 4 x 10^{-8} g/mL in vitro within 20 s was found to be 150 μg/mL. Intravaginal administration of SIF (50μg) before mating during proestrus-estrous transition phase caused complete blockage of conception in mouse model. Sub-acute genotoxicity studies in mice indicated that repetitive intravaginal application of SIF at a dose of 100μg for 14 consecutive days induced no abnormality either in length of the estrous cycle or in the morphology of the vaginal tissue. Furthermore, no adverse effect was observed on subsequent reproductive performance, neonate survival and development of pups. In addition, SIF was also found to induce immobilization in various motile bacteria in vitro viz. Escherichia coli, Pseudomonas aeruginosa and Proteus mirabilis which are known uropathogens. 6-8 h old cultures of this bacteria when incubated along with SIF caused 100% immobilization. As motility and adherence represent an integral aspect of bacterial pathogenesis, therefore, targeting bacterial motility can be exploited as a potential antibacterial therapy. Though, antibiotics have been used ever since their discovery. However, the complications involving the emergence of multi-drug resistant strains and causing chronic toxicity pose a challenge for modern medicine. Because of public concern, now the focus has shifted towards the use of safer therapeutic agents. Newer biocompatible agents including the microorganisms and their products are being examined for their potential as anti-microbials. It is suggested that SIF could be developed as a potent vaginal contraceptive as well as antibacterial agent targeting bacterial motility for future use in humans.
Cell-specific delivery of kinase inhibitors using lysozyme: A novel approach to treat renal fibrosis

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Background: Activation of renal tubular cells, mediated by several kinase pathways, is the key process in renal fibrosis. However, kinase enzymes regulate many normal physiological processes elsewhere. Therefore we developed a novel strategy to deliver kinase inhibitors selectively to renal tubular cells using lysozyme (LZM) as a drug carrier protein. This will increase therapeutic drug levels in target cells and reduce side-effects. Aim: 1) To deliver different kinase inhibitors, that is, p38 MAPK inhibitor SB202190, TGF-beta kinase inhibitor (TKI) and RhoA kinase inhibitor Y27632 to tubular cells using LZM. 2) To evaluate the efficacy of drug-LZM conjugates in renal fibrosis models in rats.

Methods: SB202190, TKI and Y27632 were conjugated to LZM using ULS™ linker. Drug-LZM conjugates were characterized for drug to protein ratio and stability in many matrices using HPLC methods. The renal uptake of the conjugates was studied upto 72h after a single i. v. injection in rats using HPLC methods. The efficacy of the conjugates was examined in either unilateral ureteral obstruction model (UUO) or ischaemia/reperfusion injury model (I/R) in rats. The antifibrotic effects were determined using RT-PCR and immunohistochemical techniques.

Results: All drug-LZM conjugates contained 1:1 drug to protein ratio. In vitro, the conjugates released drugs in kidney homogenates while remaining stable in serum and buffers. In vivo, the conjugates were rapidly accumulated in renal tubular cells and slowly disappeared at 72h after a single i. v. injection. Treatment with TKI-LZM and SB-LZM reduced renal fibrotic markers in rats with UUO and I/R injury, respectively. Intriguingly, blockade of RhoA kinase pathway with Y27632-LZM in kidneys substantially inhibited I/R-induced inflammatory and fibrotic processes. Moreover, Y27632-LZM attenuated the transdifferentiation of tubular cells after renal injury.

Conclusions: 1) We have successfully delivered several kinase inhibitors selectively to tubular cells in kidneys using our novel strategy. 2) Drug-LZM rapidly deposited in the cells and released the active drug intracellularly for prolonged time. 3) Targeted conjugates significantly inhibited renal inflammation and fibrosis in different animal models.

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Valproic Acid (VA) May Be Effective in the Treatment of Headaches Associated with Reactivation of Cerebral Toxoplasmosis (CT)

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Background: Valproic acid (VA) is effective in the treatment and prophylaxis of migraine with and without aura, refractory migraine headache, drug-resistant migraine, tension-type headache, chronic daily headache, and convulsive disorders. We found that different types of headaches were precipitated by various diseases or medications probably because marked immune irregularities associated with these events caused reactivation of CT. The aim of this study was to focus on pathomechanisms that may be responsible for these clinical effects.

Methods: Literature data were selected to illustrate that latent CNS T. gondii infection/inflammation intensity and/or host defense mechanisms may be affected by changes in production of NO, cytokines, tryptophan degradation by indoleamine 2,3-dioxygenase, limiting the availability of intracellular iron to T. gondii, production of reactive oxygen/nitrogen species, mechanisms mediated by an IFN-γ responsive gene family, and finally cause reactivation of CT. It must be added that cytosolic calcium T. gondii tachyzoite levels play an important role in their invasion to the host cells and intracellular replication.

Results: VA was found to induce generation of IL-1α, IL-1β, IFN-γ, IL-6, ROS, NO, and monocyte attractant protein-1. These irregularities could markedly affect both the host and T. gondii defense mechanisms important for immune control of the parasite, because the in vivo study showed that VA inhibited replication of tachyzoites at medium concentration of 4.1 μg/ml, similar to that of trimetoprim (5.3 μg/ml). In addition, trimetoprim exerted a synergistic effect with VA which may suggest that the mechanisms of action of these two drugs are different. This suggestion may be supported by the finding that VA is also capable of inhibiting calcium transport through cellular ion channels. Because VA brain levels in the epileptic patients are about 20% of serum concentrations, and it is known that the subjects with schizophrenia and bipolar disorder who orally receive VA therapy achieve its blood concentrations of 50-100 μg/ml, the drug may be effective also in treatment of CT.

Conclusion: VA may be effective in the treatment of CT, various types of headaches and convulsive disorders because it affects both the immune state of the host and/or the parasite, and decreases calcium ion available for T. gondii tachyzoites, important for their motility.

Clinical Importance of Antimicrobial Resistance

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Background: The World is facing the problems of growing antimicrobial resistance in almost all categories of human pathogens (bacteria, fungi, viruses and parasites), difficult to treat infections and slow drug development. The problem has become more complicated due to increase in immune compromised and critically ill patients, infected drug noncompliers, infections complicated due to other infections and emergence of new drug resistant (MDR) organisms lead to high morbidity and mortality, and increased health care costs and resources. The antibiotics development has not kept pace with the increasing trend of bacterial resistance in almost all categories of human pathogens (bacteria, fungi, viruses and parasites), difficult to treat infections and slow drug development. The problem has become more complicated due to increase in immune compromised and critically ill patients, infected drug noncompliers, infections complicated due to other infections and emergence of new drug resistant (MDR) organisms lead to high morbidity and mortality, and increased health care costs and resources.

Methods: This study is data analysis of last 5 years on antibiotic resistance in clinically important bacterial pathogens at our centre and review of literatures. Resistance was determined following standard screening, confirmatory phenotypic and/or molecular methods.

Results: Isolation of pathogenic bacteria from different anatomic sites in 2003 through 2008 ranged from 26% to 33%. The increasing trends of bacterial resistant to almost all classes of antibiotics was observed. The resistance rate to important bacterial pathogens were as follows: extended spectrum β-lactamase and AmpC β-lactamase producing Enterobacteriaceae 78%, metallo-β-lactamase producing Pseudomonas and Acinetobacter species 31% and 21% respectively, methicillin resistant Staphylococcus aureus 51%, vancomycin resistant enterococci 11%. Percent change in resistance rate for different pathogens over the period ranged from 6% to 18%. Prior use of antibiotics, presence of invasive devices, prolonged hospitalization, admission in ICUs and ventilatory support were associated with antimicrobial resistance. Mortality rate was reduced by almost 50% and hospital stay by several days in patients who received appropriate antibiotics. Mortality rate was significantly higher in patients already colonized with MDR bacteria prior to infection.

Conclusions: The trends of bacterial pathogens resistant to almost all classes of antibiotics are increasing with adverse clinical outcome. The key to address this escalating antimicrobial drug resistance problem lies in: 1) judicious use of available antibiotics, 2) avoidance of excessive and unnecessary use of antibiotics and 3) adoption of stringent infection control measures.

In vitro hypothesis, in vivo veritas. Success and failure of imatinib in cancer target therapy

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Background: Imatinib is currently registered for two major indications: (a) monotherapy in chronic myeloid leukemia (CML) and (b) monotherapy in c-Kit (CD117)–positive unresctable or metastatic gastrointestinal tumors (GISTs). Imatinib mechanism of action is to bind to specific tyrosine kinases (Bcl-Ab1 in CML and c-Kit in GISTs), thereby blocking the corresponding signaling for cell growth and proliferation of malignant cells in CML and GIST. Imatinib is an effective drug; nevertheless, resistance develops over time in many patients. Although kinase overexpression and gene amplification have been observed, the most common event in resistance is the occurrence of mutation(s) in the corresponding gene.

Methods: Clinical, biochemical and molecular modeling analyses of some important successful/unsuccessful cases observed during imatinib therapy of GIST patients are reported. Transient transfection experiments with plasmids carrying different patients KIT acquired point mutations were performed along with immunoprecipitation of total protein extracts, derived form imatinib treated and untreated cells. The molecular mechanics/Poisson Boltzmann Surface Area (MM/PBSA) computational techniques were applied to study the interactions of the wild-type and mutated receptors with imatinib at molecular level.

Results: KIT phosphorylation was detected in cells transfected with vectors carrying the specific mutant genes. Imatinib treatment demonstrated that some mutations were insensitive to the drug at all applied concentrations, while others were inhibited by imatinib, although to different extent. Modelling of the mutated receptors revealed some mutations substantially modify the protein binding pocket, thus hampering inhibitor binding, which in turn induce only relatively confined structural changes, still compatible with drug binding.

Conclusions: The results obtained from the clinical/biochemical analysis on mutated receptors testing the actual imatinib inhibition efficiency coupled with molecular modelling highlighted the streek and weakness of this inhibitor towards c-Kit mutated isoforms. Therefore these investigation ensemble could be of help in the design of new drugs and give important information to medical oncologist indicating the most suitable dose for escaping secondary resistance.

All abstracts are listed in alphabetical order of the presenting author.

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Application of Prodrug Strategies to Create Magic Bullets for the Treatment of CNS Maladies

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Background: Finding pharmacological interventions that would truly meet the definition of magic bullet in treating maladies affecting the central nervous system (CNS) has been challenging. Targets for interventions are generally not specific in the CNS and, in addition, protected from uncontrolled exposure of substances delivered into the systemic circulation by the blood-brain barrier (BBB). Although many drugs cross the BBB and exert therapeutic CNS-effects, their systemic exposure that impacts the rest of the body is unavoidable.

Methods: Strategies that, along with in vivo conversion of an inactive precursor (prodrug) to the active drug, rely on the manipulation of both the influx and efflux of substances across the BBB or, alternatively, site-specific activation of the prodrug may create magic bullets for the CNS. In this presentation, design and in vitro/in vivo evaluation of prodrugs that confine the action of neuropeptides (with focus on thyrotropin-releasing hormone, TRH) and estrogens are featured as respective examples for these strategies.

Results: For hydrophilic compounds such as peptides that practically do not cross the BBB, prodrug design has been the most versatile strategy to target them into the CNS. Simultaneously controlling activity, metabolism, transport and target-site retention via chemical manipulations is the key for creating magic bullets from neuropeptides such as TRH with promise to treat various neurological diseases (motonueron diseases, various forms of dementia and brain trauma). For lipophilic compounds such as estrogens, profound endocrine responses by off-target peripheral tissue burden therapeutic interventions for disorders of central origin that could be ameliorated by them (menopausal symptoms, impaired cognition, ischemic stroke, etc.). The success of CNS-selective estrogen therapy by a prodrug approach rests on the proper alteration of BBB-transport properties along with the facilitation of specific metabolic conversion(s) in the target tissue versus systemic bioactivation.

Conclusions: Various ways of creating magic bullets for the treatment of CNS maladies by the prodrug approach have been conceived. Although much remains to be learned about their merits, progress in the field has been steady, which clearly warrants continued exploration and development.

Acknowledgements: Supported by the National Institute of Health (grant numbers MH59360 and NS044765) and an endowed professorship (BK-0031) to L.P. from the Robert A. Welch Foundation.

Cellular Immune Toxicity of Alcohol and Cocaine: Medical Practice Based on the Evidence vs. Evidence-Based Medical Practice

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In 1906, Prof. Ehrlich shared the Nobel Prize in medicine for his pioneering research on the immune system. Largely based on his seminal work, we now realize that immune responses are complex biological processes modulated by intertwined epigenetic, genomic, molecular, biochemical, cellular and physiologic regulatory mechanisms. When acting in concert and appropriately, these processes converge toward efficient, rapid and complete removal of the invading pathogens. Immune events are delivered by several cell populations that work in conjunction with soluble factors, including antibody species that recognize and target individual pathogens. Prof. Ehrlich termed this remarkably specific process the "magic bullet". Our understanding of how immunity works has improved significantly over the past decades, and the "magic bullet" theory is today the prevalent model for treatment intervention in infectious diseases, cancer, oral pathologies, and even certain neurodegenerative pathologies (e.g., Alzheimer's disease). Illicit drugs of abuse, often co-abused with alcoholic beverages, interfere significantly with magic bullet-driven treatment interventions. Reports describe the many benefits of detoxification programs, but do not concur with respect to immune ameliorations.

The contemporary model of clinical intervention is grounded on the systematic evaluation of the best available evidence, and the judicious integration of appropriately revised clinical practice guidelines in the evidence-based treatment decision-making process. We present a systematic review of the available evidence with respect to the immunotoxicity of alcohol and joint alcohol/cocaine abuse, and of the immune benefits of detox programs, followed by level of evidence analysis, acceptable sampling analysis and meta-analysis. We demonstrate the practical usefulness of our findings in the context of the novel evidence-based model for XII Century medical practice.

All abstracts are listed in alphabetical order of the presenting author.
Virus-Like Particles As Magic Bullets For Immune System

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The development of genetic engineering techniques in the 1970s offered a broad range of applications, which were immediately followed by the expression of viral and non-viral genes in efficient heterologous expression systems, first of all, in bacteria and yeast. Special attention has been devoted to the synthesis of viral structural proteins as constituents of viral capsids and envelopes with their subsequent spontaneous self-assembly into correctly organized virus-like particles (VLPs). Food and Drug Administration's (FDA)-approved vaccines against hepatitis B and human papilloma viruses represent genetically engineered VLPs generated in heterologous expression systems. VLP-based vaccines are also being developed against malaria, HIV/AIDS, hepatitis C, human and avian influenza, as well as against many other infectious and non-infectious diseases. Moreover, VLPs from almost all classes of viruses are being evaluated now or have just been adopted to use as a scaffold for presentation of foreign immunological epitopes on their surface. VLP technologies possess obvious advantages for the generation of safe and efficacious prophylactic and therapeutic vaccines. First, the repetitive antigenic structure of VLPs makes them highly immunogenic. Second, chimeric VLPs are lading viral genomes or genes and are non-infectious, although they are mimicking infectious viruses in their structural and immunological features. Third, VLPs are generated by highly efficient heterologous expression of the cloned viral structural genes with subsequent quantitative in vivo or in vitro self-assembly of their products. Fourth, VLPs can be obtained by simple and efficient purification procedures. A broad range of viral structural proteins is able to form autologous VLPs consisting solely of structural protein(s) of the target virus. Many of them have been tested successfully for the construction of chimeric VLPs retaining their VLP-forming ability, but carrying foreign epitopes. VLP technologies allow the generation of (1) uniform chimeric VLPs consisting of identical fusion protein subunits, (2) mosaic VLPs consisting of carrier and fusion protein subunits, and (3) pseudotyped VLPs consisting of non-fused autologous and foreign proteins. VLPs can be used for a broad range of applications, including nanotechnology, but first of all for vaccine development, as magic bullets for immune system.

EHRlich II – 2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich
Nürnberg, October 3-6, 2008

Discovery of the HCV NS3/4A Protease Inhibitor, Bocceprevir (SCH503034).
Key Steps in Structure-Based Optimization.

PRONGAY AJ

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The Hepatitis C Virus (HCV) infects about 200 million people worldwide. The current combination therapy of pegylated-interferon-α and ribavirin gives a 70-80% sustained virological response against most genotypes, but only 40% against genotype 1. Finding a more effective anti-HCV treatment has been a major objective of pharmaceutical companies for the past decade. In an effort to find a “Magic Bullet”, a number of the enzyme activities of HCV have been targeted. The approach undertaken at Schering-Plough Research Institute has been to use structural information to assist the more traditional assay-based methods to move compounds that inhibit viral enzyme activity from a “Hit” to a “Lead” state, then through optimization into a clinical candidate. This research paradigm has resulted in an inhibitor of the HCV NS3 protease entering Phase III Clinical studies. An overview of this Structure-Based Drug Design process will be presented.

The Potential of Orally Presented Mistletoe Lectins in Cancer Therapy.

PRYME IF

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Background: Hitherto mistletoe preparations have been essentially administered by subcutaneous (s.c.) injection. This route, however, has clear limitations. Evidence now shows that a preparation containing mistletoe lectins (MLs) can be presented orally achieving the same results as s.c. administration. Being heavily glycosylated MLs can be taken orally since they are both resistant to low pH in the stomach and are unaffected by proteolytic enzymes.

Methods: Experiments were performed on female NHR mice. Mice were initially maintained on a standard pelleted diet with free access to water. Groups of five mice were fed for 3 days on a Lactalbumin based, semi-synthetic diet (La). All mice were then injected s.c. with 2 x 10^7 Krebs II Non-Hodgkin’s Lymphoma (NH) tumour cells. One group was switched to an ML-1 diet (lectin concentration: 1.7mg/kg diet) a second (control) group was continued on the La diet. After 11 days animals were sacrificed and solid s.c. tumours were excised, weighed and prepared for histological examination.

Results: Following binding to specific receptors ML-1 induces a biological response. Immunomodulatory effects are initiated resulting in both activation and an increase in the population of natural killer cells. Many NHL cells enter the apoptotic pathway. An anti-angiogenic response severely curtails tumour growth. An interesting property of ML-1 is modulation of tumour characteristics seen as an increased level of differentiation.

Conclusions: In addition to the effects mentioned in the Results section ML can exert a cytotoxic effect on sensitive cells (e.g. cancer cells) bearing surface receptors to which the B-chain can bind. On internalization of the A-chain, and its activation, a RIP effect (ribosome-activating protein) is exerted leading to an induction of apoptosis, culminating in tumour cell death. An advantage of using the oral route is that MLs can come into direct contact with tumours that are localized in the oral cavity, oesophagus or gastro-intestinal tract and thus be able to exert a direct cytotoxic effect on tumour cells. A further advantage of using the oral route is that injection is avoided and the involvement of health personnel is thus unnecessary. It is proposed that the use of an oral mistletoe lectin-containing preparation would provide an excellent alternative, or supplement, to conventional forms of cancer treatment.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts Page A-262
**Molecularly Imprinted Polymers (MIPs) for the Drug Targeting**

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**Background:** Specific molecular recognition is a fundamental requirement of living systems and, through millions of years and countless rounds of evolutionary optimization, biology has become a master of the art. At the cellular and sub-cellular level the fundamental processes of life, information transfer and reaction catalysis, rely on the specific interaction of low molecular weight molecules with macromolecular hosts. In the majority of such events the macromolecule is a protein. Processes as diverse as neural transmittance, respiration, immune defense, cellular differentiation and nutrition all rely on the basic principle of specific molecular recognition. It is therefore not surprising that scientists have invested huge amounts of time and effort, initially into harnessing the potential of biological molecular recognition, antibodies and enzymes, and more recently in trying to mimic these properties in synthetic materials. One of the most promising of these ones is molecular imprinting.

Molecular imprinting is a very useful technique for incorporating specific substrate recognition sites into polymers. The molecular recognition characteristics of these polymers are attributed to the complementary size, shape, and binding sites imparted to the polymers by the template molecules.

The concept of molecular imprinting has a long history dating back to the early 1930s. However, the preparation of organic polymers with molecular recognition was first reported only in 1972, initiating the molecular imprinting technology as it is known today. Molecular imprinting has now become an established method and has also been applied in the areas of synthetic chemistry and analytical chemistry. MIPs have been used as chromatographic stationary phases for enantiomeric separations, and for solid-phase extraction, catalysts and sensor design, as well as for protein separation, as receptor, antibody and enzyme mimics and recently Drug Delivery Systems too.

But it is perhaps in the area of drug delivery, in particular ‘intelligent drug release’ and ‘magic bullet’ drug targeting, that significant future opportunities lie. Although relatively few studies have been reported in the literature about the intelligent drug delivery and targeting, they represent an important starting point for the development of new generations of intelligent and self-regulated drug delivery systems.

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**Steroid Sulfatase Inhibitors – Novel Therapeutic Agents for Hormone Dependent Cancers**

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**Background:** Inhibition of steroid sulfatase (STS), the enzyme responsible for the hydrolysis of steroid sulfates, represents a potential novel treatment for postmenopausal women with hormone-dependent breast cancer. Estrone and DHEA are formed by the sulfatase pathway and can be converted to steroids (estradiol and androstenediol, respectively), which have potent estrogenic properties.

**Methods:** STX64, a tricyclic coumarin-based sulfamate that irreversibly inhibits STS activity, was selected for the first in class Phase I trial of a STS inhibitor in postmenopausal women with breast cancer. STX64 was administered orally (nine patients at 5mg and five patients at 20mg) as an initial dose followed 1 week later by 3 x 2 weekly cycles, with each cycle comprising daily dosing for 5 days followed by 9 days off treatment. Blood and tumor tissue samples were collected for the assessment of STS activity and serum was obtained for steroid hormone measurements before and after treatment.

**Results:** The median inhibition of STS activity by STX64 was 98% in lymphocytes and 99% in breast tumor tissue at the end of the 5-day dosing period. Serum concentrations of estrone, estradiol, androstenediol and DHEA all decreased significantly from pretreatment levels. Unexpectedly, androstenedione and testosterone concentrations also decreased. Four patients, all of whom had previously progressed on aromatase inhibitors, showed evidence of stable disease for 2.75 to 7 months. The drug was well tolerated.

**Conclusions:** STX64 is a potent, well tolerated STS inhibitor which causes significant decreases in serum concentrations of estrogenic steroids. Conversion of second generation STS inhibitors to Magic Bullets will be highlighted.
Synthesis Of Fluorescent Heteroaromatic Compounds Using Dehydroamino Acids As Building Blocks, Studies Of DNA And Biomembranes Interactions. Evaluation Of Antiproliferative Effects On Tumor Cell Lines

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Background: The synthesis of new anticancer agents is an important goal. Aims: 1) To synthesize new fluorescent compounds, a 3-(dibenzothien-4-yl)indole (1), a 3-(benzothien-3-yl)benzothieno[2,3-b]pyrrole (2) and a 3-(benzothien-2-yl)benzothieno[3,2-b]pyrrole (3). 2) To study their interaction with DNA and liposomes. 3) To evaluate their effects in tumor cell lines.

Methods: Absorption and fluorescence spectrocopies were used to study their photophysical properties in different solvents and their interactions with salmon sperm ds-DNA including fluorescence quenching experiments with iodide ion. Their interaction with liposomes of dipalmitoyl phosphatidylcholine (DPPC) prepared by injection, was studied by fluorescence. The antiproliferative effects on tumor cell lines of breast adenocarcinoma (MCF-7), glioblastoma (SF-268) and non-small cell lung cancer (NCI-H460) were evaluated after a continuous exposure of 48h, using the protein-binding dye sulforhodamine B. Results represents means ± SEM of 3 exp. performed in duplicate.

Results: Compounds 1-3 were synthesized in good yields. In the fluorescence spectra a red shift in the \( \lambda_{em} \) (nm) is observed from apolar to polar solvents. In the fluorescence spectra using increasing [DNA]/[compound] ratios an increase in the emission intensity is observed. The fraction of molecules accessible to iodide ion was very low. In DPPC liposomes (25 °C) the emission spectra are very similar to the ones in cyclohexane. The results of the antiproliferative effects are shown below.

<table>
<thead>
<tr>
<th>Compound</th>
<th>GI_{50} (µM)</th>
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<tr>
<td></td>
<td>MCF-7</td>
</tr>
<tr>
<td>1</td>
<td>11.00±0.60</td>
</tr>
<tr>
<td>2</td>
<td>7.88±0.08</td>
</tr>
<tr>
<td>3</td>
<td>19.10±1.50</td>
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</tbody>
</table>

Conclusions: 1) The preferred mode of binding with DNA is the intercalation. 3) Their location in liposomes of DPPC is the hydrophobic region. 4) A good to high inhibitory effect on the growth of the tested cell lines was observed. Compound 3 shows a high specificity for the NCI-H460 cell line.

Thanks are due to the Fundação para a Ciência e Tecnologia (FCT, Portugal) and FEDER to financial support through the research centres, the research project POCI/59407/2004 and pos-Dir grants attributed to A.S.Abreu (SFRH/BPD/24538/2006) and to L.V.-S. (SFRH/BPD/29115/2006).
Synthesis And Biological Evaluation Of Some New 2-Cinnamimidobenzamides As Potential Antagonists Of The HDM2-P53 Protein-Protein Interactions

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Background: Stomal infection (SI) following PEG is commonly due to bacteria coming from oro-pharynx and or skin surface. We hypothesise that by combining parenteral antibiotic with local anti-septic spray reduces the incidence of stomal infection by reducing the bioburden at the skin surface.

Methods: Total 96 patients. Group A (n=34) M:F 18:16, mean age (MA) 74. Group B (n=28) M:F 16:12, mean age (MA) 78. Group C (n=34) M:F 14:20, mean age (MA) 76. At midweek and end of week 1. Prophylaxis with 1:

- 3) Understanding the physics outcome. 2) Inadequate dosing is a serious issue in therapeutics that requires model key determinants of molecular transport within and into brain tissue using inputs from in-vivo imaging. 2) From this, obtain time-and-space dependent maps of molecular motion in response to therapeutic interventions. 3) Use this to translate and analyze into optimal delivery protocols and devices for application by a range of health care providers as well as drug development.

Methods: We developed transport models, solution methods, and estimates of key parameters in the equations, for individuals from in-vivo imaging. Interstitial transport is validated using radiotransport contrast agents, and transcapillary transport via microdialysis. Direct delivery in tissue was studied in six pigs with concentration of a contrast agent measured with new quantitative imaging, and in seven humans using radioactive iodine. Monkey studies are ongoing. Systemic delivery is studied using microdialysis in humans.

Results: 1) Modeling and display suggest alterations of widely accepted therapy protocols and device placement, e.g. in brain cancer, we propose earlier administration of temodar (temozol) and entirely new catheters and placement (pentamural infusions). 2) The extracellular spaces can expand to more than double their resting value in response to disease-induced edema or therapeutic infusions, having a major impact on flow of fluid and particulates in brain. 3) Determinants of transport can be quantified by specialized, but clinically acceptable, in-vivo imaging.

Conclusions: 1) Patient-specific estimation of distribution of therapeutics can be valuable in maximizing the chance of success of clinical trials, and in therapeutic outcome. 2) Inadequate dosing is a serious issue in therapeutics that requires collaboration of diverse disciplines and technologies. 3) Understanding the physics (poroelasticity, microhydrodynamics) of the brain is an important companion to pharmacology in getting drugs to desired locations.

Authors' disclosure statement:The experiments were conducted by collaborators at the Virginia Commonwealth University (pigs), Duke University (humans with radioactive markers), the Johns Hopkins University (humans with microdialysis), and the University of California in San Francisco (monkeys). The collaborators will be named in the talk. The work was supported in part by BrainLAB AG, which has a financial interest in the software developed in the course of the studies.

Breaching The Barriers Of The Brain: From Physics To Cures

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Therataxis LLC, Baltimore, USA

Background: Getting the right dose to the right target is critical to any form of delivery for brain therapies, and the blood brain barrier is central to regulating this. In individuals, diseases and therapies modify key factors of molecular motion, thus complicating the problem. We model the physics of brain to predict response to new protocols, devices, and therapeutics. Delivery methods range from systemic delivery of small molecules, to direct injection of large ones into tissue. Aim: 1) Model key determinants of molecular transport within and into brain tissue using inputs from in-vivo imaging. 2) From this, obtain time-and-space dependent maps of molecular motion in response to therapeutic interventions. 3) Use this to translate and analyze into optimal delivery protocols and devices for application by a range of health care providers as well as drug development.

Methods: We developed transport models, solution methods, and estimates of key parameters in the equations, for individuals from in-vivo imaging. Interstitial transport is validated using radiotransport contrast agents, and transcapillary transport via microdialysis. Direct delivery in tissue was studied in six pigs with concentration of a contrast agent measured with new quantitative imaging, and in seven humans using radioactive iodine. Monkey studies are ongoing. Systemic delivery is studied using microdialysis in humans.

Results: 1) Modeling and display suggest alterations of widely accepted therapy protocols and device placement, e.g. in brain cancer, we propose earlier administration of temodar (temozol) and entirely new catheters and placement (pentamural infusions). 2) The extracellular spaces can expand to more than double their resting value in response to disease-induced edema or therapeutic infusions, having a major impact on flow of fluid and particulates in brain. 3) Determinants of transport can be quantified by specialized, but clinically acceptable, in-vivo imaging.

Conclusions: 1) Patient-specific estimation of distribution of therapeutics can be valuable in maximizing the chance of success of clinical trials, and in therapeutic outcome. 2) Inadequate dosing is a serious issue in therapeutics that requires collaboration of diverse disciplines and technologies. 3) Understanding the physics (poroelasticity, microhydrodynamics) of the brain is an important companion to pharmacology in getting drugs to desired locations.

Authors' disclosure statement:The experiments were conducted by collaborators at the Virginia Commonwealth University (pigs), Duke University (humans with radioactive markers), the Johns Hopkins University (humans with microdialysis), and the University of California in San Francisco (monkeys). The collaborators will be named in the talk. The work was supported in part by BrainLAB AG, which has a financial interest in the software developed in the course of the studies.

Identification Of Novel Hepatitis C Virus Polymerase NS5B Inhibitors Through Structure-Based Virtual Screening And 3-D QSAR Studies

RAGNO R1, MUSMUCA I1, CAROLI A1, SIMEONI S1, MAI A1, KRISHNAN R2, KAUSHIR-BASU N2

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Background: Although a great deal of research has been focused on the development of anti-HCV agents, to date no vaccine is available and there is no effective therapy for all genotypes. Thus, there is an urgent need to identify and develop HCV-specific antiviral agents to improve the effectiveness of actual HCV therapy.

Nonstructural protein 5B (NS5B), a 66 Kda RNA-dependent RNA polymerase (RdRp) has attracted a great deal of focus as a target for drug development since it plays a pivotal role in HCV replication. 3) Methods: Here we present the development of structure-based 3-D QSAR models for inhibitors binding either at the thumb or the palm NS5B allosteric sites. The final models proved to be statistically robust showing q2 and r values in the range of 0.5-0.9. The use of external test sets showed good predictive abilities of the 3-D QSAR models.

Through either ligand based (Surflex) or structure based (Autodock) molecular alignment the NCi Diversity Set was then virtually screened and the result were externally scored with the 3-D QSAR models. For each allosteric site, the first 20 molecules predicted more active were selected for biological assay against HCV. Results: Preliminary biological data proved that among the selected compounds three derivatives showed to be effectively active against NS5B at a fixed dose of 100 µM with percentage of enzyme inhibition in the range of 60-70%. Details of computational and virtual screening procedures will be reported along with further biological investigations.

Thymus Binding Site Palm Binding Site
The Immunorepressive Drugs- Cyclosporin A, FK506 And Rapamycin

The Herpes Simplex Virus (HSV) Vaccines: Old Problems, New Challenges

Prevalence And Characteristics Of Verotoxigenic Producing Escherichia coli O157:H7 Isolated From Goats And Cattle Carcasses In Tanzania

Authors' disclosure statement. Introduction of new adjuvants, which shift the cytokine production towards the stimulation of helper T-cells indicates a promising development. Even when the immunotherapeutic use of HSV vaccine is effective only partially, it still might represent an alternative to chronic chemotherapy of recurrent labial and/or genital herpes.

Culture, Susceptibility Testing And Genotyping Of Mycobacterium Tuberculosis Isolated From Tuberculosis Patient In Bangladesh

Background: It is essential to study the magnitude of the burden of tuberculosis (TB) in order to control the disease efficiently. Aims: 1) To isolate M. tuberculosis from sputum samples of TB patients, 2) Susceptibility testing of isolates and 3) To record circulating phylogenetic clades of M. tuberculosis.

Methods: M. tuberculosis was isolated from the pulmonary TB patients of selected rural and urban areas of Bangladesh. Conventional method was followed to isolate the pathogen from sputum samples and to tests susceptibility with respect to first anti-tub drug. Strains were genotyped by spoligotyping technique. Phylogenetic clade designation was performed matching the spoligotypes with that available in the International Database: SpolDB4.

Results: This study included susceptibility testing of 667 isolates. Resistance to isoniazid, rifampicin, streptomycin and ethambutol was 14%, 6%, 45%, and 8% respectively. Simultaneous resistance to isoniazid and rifampicin was detected in 6% of the isolates. Randomly selected 224 strains were genotyped by spoligotyping. One hundred and ninety three (86%) of 224 isolates were grouped into 31 patterns and 31 pattern isolates (14%) were unique. The comparison of spoligopatterns with SpolDB4 indicates that 75% of M. tuberculosis population of this study composed of Principal Genetic Group 1 (PGG1) having clades like; East African Indian (EAI, 44%), Beijing (15%), and the Central-Asian (CAS, 15%). The remaining 25% of the isolates belonged to PGG 2 and 3 having Latin-American-Mediterranean (LAM) clade as predominant. A new pattern was signature was detected in 49 out of 224 isolates within the clade EAI (EAI 6 BGD1) and was named Matlab type after the name of the field site where it was isolated for the first time.

Conclusion: Drug resistance was significantly higher among patients previously received anti-TB treatment. Besides, this study provides a first description of the genetic population structure of M. tuberculosis in Bangladesh, where TB patients are infected with a diverse and heterogeneous population of M. tuberculosis without predominance of a single genotype. The newly described Matlab types are suggestive of new South or South-East Asian-linked emerging genotypes.

The Immunorepressive Drugs- Cyclosporin A, FK506 And Rapamycin

Modulate The Functional Expression Of The Na+-Ca2+ Exchangers In An Isolated Specific Manner

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Background: Treatment of organ transplant patients with immunorepressive drugs leads to complications such as hypertension, nephrotoxicity, neurological symptoms and bone loss that can be linked to impaired cell Ca2+. The Na+-Ca2+ exchanger is a major cell Ca2+ regulating protein encoded by three genes: NCX1, NCX2 and NCX3. NCX1 protein is expressed ubiquitously and NCX2 and NCX3 are expressed almost exclusively in the brain. We have investigated the modulation of NCX expression by the immunorepressive drugs Cyclosporin A, FK506 and Rapamycin and the non-immunorepressive PSC833. The drugs were added to the cultured cells.

Methods: Functional expression of NCX protein was measured in transfected HEK 293 cells and non-transfected NCX1-expressing HEK2 cells. Na+-dependent Ca2+ fluxes, surface and total NCX protein expression were determined in parallel by FACS analysis, surface biotinylation, Western analysis and quantitative PCR. siRNA targeting Cyclophilin A was used for its knock down. All measurements were done in triplicates and repeated at least 5-7 times.

Results: Treatment of NCX1, NCX2 and NCX3 transfected HEK 293 cells with Cyclosporin A and PSC833 results in down regulation of surface expression and transport activity of the protein without a decrease in expression of cell NCX protein, whereas CsA had no effect on total cell NCX1 protein expression. PSC833 reduced mRNA and cell protein expression of NCX2 and NCX3. FK506 had no effect on NCX1 expression yet it down-regulated NCX2 and NCX3 surface expression and transport activity without any apparent effect on cell NCX expression. Rapamycin had no effect on NCX2 and NCX3 protein expression yet it reduced NCX2 and NCX3 transport activity. Knock-down of Cyclophilin A modulated NCX1 expression and the effect of Cyclosporin A.

Conclusions: Since all the experimental conditions in our studies were identical, presumably the different drug response is related to structural differences between NCX isoforms. Expression of NCX genes is tissue specific.

Prevalence And Characteristics Of Verotoxigenic Producing Escherichia coli O157:H7 Isolated From Goats And Cattle Carcasses In Tanzania

RAJ MA

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The prevalence of Verotoxigenic producing Escherichia coli (VTEC) in cattle and goats carcases were investigated between September 2002 and December 2003 by culturing and immunomagnetic separation technique. A total of 167 Escherichia coli colonies from carcases of cattle (300), and goat (263), from Morogoro and Dar-es-salaam were isolated in this study. VTEC O157 strains were recovered from 17 (5.67%) cattle carcases and none from goats. Of 167 E.coli strains, 17 were grouped into sorbitol non-fermenting and gentamicine positive and 29 strains were sorbitol positive and gentamicine positive. The remaining 38 were sorbitol negative and gentamicine positive.V using Reversed passive latex agglutination kit from Denka Japan indicated that all isolates produced verocytotoxin. Further characterization of the VTEC isolates showed that 1(4%) of the bovine VTEC strains was positive only for stx1. Stx2 gene alone was detected in 4(20%) of bovine isolate. Both stx1 and stx2 gene were present in one (4%) of bovine isolates. Eae A was detected in 4 (20) of bovine isolates. Stx 1, stx2 eae A and Ehy A were present in one (4%) bovine isolates. Other bacterial agents such as Salmonella spp. Proteus spp and coliforms were also isolated. The VTEC O157 strains were resistant to gentamicine, chloramphenicol, streptomycin, and amoxiclav. This study is the first attempt to investigate the prevalence of VTEC O157 in goats and cattle carcases in Tanzania. Cattle carcases are contaminated with verotoxigenic Escherichia coli O157:H7 in this region.

The Herpes Simplex Virus (HSV) Vaccines: Old Problems, New Challenges

RAJCAI J

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Background: The HSV candidate vaccines tested until now were either purified subunit vaccines or recombinant envelope glycoproteins. In mice, guinea pigs and rabbits immunized with a classic subunit vaccine (Rajčaní, J et al. Acta virol. 39, 1995, 137-40), clearance protection against viral challenge and reduction of the extent of latency were demonstrated.

Methods: To compare our experimental HSV vaccines (based on the purified extract and on the recombinant gD polypeptide), Balb/c mice were immunized with the recombinant fusion protein gD1/313 (FpgD1/313, the ectodomain of gD1), with the non-pathogenic ANGpath gE-del virus, with the purified cell extract and with a plasmid (pcDNA3.1-gD) expressing gD1.

Results: Resistance against virulent virus challenge was found after immunization with the pcDNA3.1-gD plasmid and with the gD1-vec virus. A medium grade, but still satisfactory protection was noted following immunization with the FpgD1/313. The non-pathogenic ANGpath gE-del virus, with the purified cell extract and with a plasmid (pcDNA3.1-gD) expressing gD1.

Conclusion: The classical purified subunit vaccine (especially in combination with a novel adjuvant), might be a more powerful immunogen than a single recombinant glycoprotein.

Abstracts
Polymyxin B Sulphate: A Brief Overview

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Background: Polymyxin B sulphate, an antibiotic discovered in 1947, is used in the treatment of infections caused by gram-negative bacteria, particularly Pseudomonas aeruginosa and Escherichia coli. They are decapetides with antimicrobial spectrum that includes gram-negative bacteria, prominently Pseudomonas aeruginosa. Many questions remain unanswered about polymyxins since most of the research was carried out before the 1980s and the methods for evaluation of the antibiotic were not as advanced as today. Therefore, a detailed investigation on the polymyxin production by Bacillus polymyxa is warranted. Methods: Optimization of nutritional and physiological parameters for high yield of polymyxin B sulphate production was done at shake flask level. The process was scaled up to 10 L in a bioreactor. A process for purification of the antibiotic was developed. Results: By optimization, a yield of 2 g/L of polymyxin B sulphate could be obtained. The process was scaled up and validation at 10 L level in a bioreactor. Downstream processing for isolation of the antibiotic involved steps including charcoal adsorption, chromatographic separation on Amberlite resin and solvent precipitation. A recovery of 33% could be obtained. The process was scaled up and validation at 10 L level in a bioreactor. Conclusions: A detailed study for optimized production of polymyxin B sulphate was done. The downstream process to isolate the antibiotic from the broth was developed.

Authors’ disclosure statement: Some of the data being in the patenting stage cannot be presented.

Ciprofloxacin Resistance Profile In Klebsiella Pneumoniae Isolates During 2002-2007 In Paediatric Septicemic Cases Of A Tertiary Care Hospital In India

RATH B, LAIMER T, VYHNANEK P, ROTH-MANGEI S, MAURER W, POLLAK A
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Background: Recent trials have confirmed the safety of recommended vaccines, but rumours surrounding the safety of human vaccines spread quickly via the international media causing confusion and anxiety among patients, parents and physicians alike. Methods: In view of these concerns, The Vienna Vaccine Safety Initiative (VVi; http://www.meduniwien.ac.at/vivi/vivi/) has been founded in 2008 as an international scientific forum aiming to promote evidence-based vaccine safety research and communication. Results: Initial projects are dedicated to the role of the media in vaccine risk communication, and to perceptions of vaccine safety among vaccine recipients as well as providers. The goal is to generate feedback for the scientific community. Interim Results of a study assessing vaccine safety perceptions among parents in Vienna will be presented along with a survey of vaccine safety training and awareness among pediatricians in collaboration with the International Pediatric Association (IPA). Conclusion: The Vienna Vaccine Safety Initiative has been formed to address the urgent need to promote vaccine safety training and research. Additional projects will serve to generate awareness of international vaccine safety standards in the public as well as pilot projects in collaboration with international vaccine safety experts from the developed and developing world. It is hoped that the activities of this new initiative will be able to generate concepts for evidence-based action towards accurate AEFI reporting while strengthening trust in vaccines.

The Vienna Vaccine Safety Initiative (VVi) – An International Scientific Forum Aiming To Promote Evidence-Based Vaccine Safety Research And Communication

RATH B, LAIMER T, VYHNANEK P, ROTH-MANGEI S, MAURER W, POLLAK A
Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Austria

Background: Recent trials have confirmed the safety of recommended vaccines, but rumours surrounding the safety of human vaccines spread quickly via the international media causing confusion and anxiety among patients, parents and physicians alike. Methods: In view of these concerns, The Vienna Vaccine Safety Initiative (VVi; http://www.meduniwien.ac.at/vivi/vivi/) has been founded in 2008 as an international scientific forum aiming to promote evidence-based vaccine safety research and communication. Results: Initial projects are dedicated to the role of the media in vaccine risk communication, and to perceptions of vaccine safety among vaccine recipients as well as providers. The goal is to generate feedback for the scientific community. Interim Results of a study assessing vaccine safety perceptions among parents in Vienna will be presented along with a survey of vaccine safety training and awareness among pediatricians in collaboration with the International Pediatric Association (IPA). Conclusion: The Vienna Vaccine Safety Initiative has been formed to address the urgent need to promote vaccine safety training and research. Additional projects will serve to generate awareness of international vaccine safety standards in the public as well as pilot projects in collaboration with international vaccine safety experts from the developed and developing world. It is hoped that the activities of this new initiative will be able to generate concepts for evidence-based action towards accurate AEFI reporting while strengthening trust in vaccines.

All abstracts are listed in alphabetical order of the presenting author.

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Acetaminophen: The Global Pain Killing Magic Bullet Of The New Millennium

RAY SD, BULJUKI E, ZINKOVSKY D, LAHOTI T

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Background: For decades now, acetaminophen (APAP) has enjoyed untainted popularity as the most effective and possibly the safest over-the-counter analgesic of the world. It has maintained its prestige by itself and in combination with several other drugs such as morphine, codeine, aspirin etc., which has mesmerized the field of medicine as the ‘Magic Bullet for Pain Management’. APAP uses COX1/COX2/COX-3 as instruments to subdue pain, but unfortunately its safety and efficacy overshadow its potential to cause hepatotoxicity. Our laboratory has used APAP as a model toxin for decades to understand mechanisms of cell injury and cell death, and we were the first to report its potential to induce genomic injury and apoptotic cell death in the liver. APAP is unique because of its ability to communicate with various macromolecules and all the cellular components (membrane, ER, mitochondria, nucleus). CYP450-mediated biotransformation of APAP produces a highly reactive intermediate. NAPQI, and cytotoxicants such as ROS, RNS & ROS-RNS hybrids. Concerted actions of all these dramatically deplete intracellular antioxidants, induce massive oxidative stress and provide a platform to initiate molecular changes that orchestrate various forms of cell death in the liver.

Methods: This study (/ICR mice) was designed to explore some of the molecular mechanisms after exposure to toxic doses of APAP (500 mg/Kg. ip) for 0 - 24 hours. Livers were analyzed for tissue biochemistry, ultrastructural changes & differential expression of pro- and antipatogenic genes.

Results: APAP induced massive liver injury (ALT) coupled with massive oxidative stress (lipid peroxidation) and genomic injury (12DNA fragmentation). Gene expression studies revealed considerably reduced anti-apoptotic genes (bcl-2, bcl-XL) and elevated levels of pro-apoptotic genes (bcl-Xs, p53). Ultrastructural studies revealed megamitochondria formation and evidence of cytochrome c leakage a unique hallmark of mitochondrial route of apoptosis.

Conclusion: In addition to the classic apoptotic markers, this in vivo study clearly suggests APAP’s ability to use the mitochondrial cytochrome c-release signaling system as a unique mechanism to propel this cell suicidal process. Since this pathway is maneuverable, it may have tremendous therapeutic implications in liver injury management.

Lapatinib: New Expectations

RAZIS E

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In the recent years there has been substantial improvement in the management of Her2Neu overexpressing breast cancer. The addition of trastuzumab, a humanized anti Her2 monoclonal antibody to the treatment of both metastatic and early disease has resulted in impressive amelioration of Disease-Free survival and overall survival for these patients. However a significant number of Her2Neu overexpressing tumors have either primary resistance or develop it after response to trastuzumab. The mechanisms of resistance are varied and are the topic of extensive research, but it is clear that new molecules are needed for the management of refractory cases.

Lapatinib is a small, orally administered, reversible dual Erb1 and Her2 inhibitor. It is a tyrosine kinase, which inhibits Erb phosphorylation and survivin thus inducing apoptosis. Lapatinib inhibits downstream signaling from the hetero- and homodimerization of EGFR, Erb2 and possibly other Erb family members, thus preventing or delaying the development of resistance.

Original phase I studies showed that lapatinib is active in nearly pretreated Her2 positive metastatic breast cancer with a median response duration of 5.5 months. Most frequent drug related event included diarrhea (42%) rash (31%) nausea (13%) pruritus and fatigue (10%). The development of rash did not seem to correlate with response. The optimally tolerated dose was 150mg/day. Subsequent phase II trials at that dose level yielded a response rate of 48% in heavily pretreated patients but in the first line therapy of FISH positive patients the DRR was 28%.

The next study was a randomized phase III trial in Her2 positive pretreated patients who received either capecitabine 2500mg/m²/day or lapatinib 1250mg/day. In this study median time to progression and progression free survival were statistically significantly longer in the combination group. Furthermore some responses were seen in CDS disease. Additional studies in this area reveal modest responses in CDS metastasis.

Further ongoing studies of lapatinib efficacy include randomized trials in combination with other chemotherapy agents (such as the taxanes) combination with trastuzumab for complete Her2 signaling blockade and combinations with hormonal therapy (tamoxifen). Also ongoing are trials in the neoadjuvant adjuvant setting and in CNS disease. Extensive investigation into the mechanism of resistance to Her2 blockade is also in progress specifically into molecular determinants of resistance such as PTEN, IGF-1R and akt and into the best method for identification of responders.

Last but not least a clear grasp of the cardiac toxicity associated with lapatinib is necessary. Because er2 signaling is important for cardiac function there were concerns that lapatinib had the potential of cardiac toxicity.

The incidence of symptomatic and asymptomatic decrease in LVEF among the 1674 patients treated with lapatinib in the phase III trial was only 1.3% and the drop was reversible but ongoing studies are in progress.

Finally, ongoing studies in other Her2 overexpressing solid tumors are also in progress.
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Uncovering Tumor Systems Biology By Biomodulatory Therapy Strategies

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How can we structure therapies in metastatic cancer as a source for detecting tumor-associated systems-biological processes as adjustable sizes available for biomodulatory therapies? A therapy-derived methodological approach to explore tumor-associated systems biology should be explicated and developed by means of analyses of recently published biomodulatory therapy approaches involving combined immunomodulatory, anti-inflammatory and angiostatic therapy in the treatment for advanced chemoresistant tumors of quite different origin. Biomodulatory therapy approaches in tumors intend to develop systems-terms that provide a basis for broadening therapy-relevant capacities by regulating biological systems processes for tumor control. Combined targeted therapies of tumor-associated wound healing mechanisms, namely inflammation and neoangiogenesis, have shown that – using an approach for understanding systems biology as adjustable size – we may break through the barrier of complexity of tumor-stroma-interactions in a therapeutically relevant way.

Digoxin plasma concentrations of four different dosing schedules commonly used in clinical settings.

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3 Laboratorio Clinico Diagnostico, Concepcion, Chile

Background: The different therapeutic schedules used for the prescription of digoxin have little theoretical support and are based mainly on the patients clinical response. The aim of this study was to measure digoxin plasma levels in patients using four different prescription schedules.

Methods: A total of 175 patients were included in this study with previous written consent. Inclusion criteria: patients who had been prescribed Digoxin for at least 1 month prior to the study. Four groups of patients were studied. Group I (n=56): digoxin 0.25 mg/day, from Monday to Friday.

Group II (n=30): digoxin 0.25 mg/day, from Monday to Saturday.

Group III (n=53): digoxin 0.25 mg/day, from Monday to Thursday.

Group IV (n=36) digoxin 0.125 mg/day continuously.

Plasma digoxin levels were measured in two consecutive mondays, before taking the daily dose of the drug, serum creatinine and creatinine clearance was calculated. Therapeutic plasma concentration range was set between 0.5 and 2 ng/ml. Digoxin was determined using immune analyses through a flurometric enzyme.

Results:

<table>
<thead>
<tr>
<th>Results</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Patients</td>
<td>56</td>
<td>30</td>
<td>53</td>
<td>36</td>
</tr>
<tr>
<td>Age</td>
<td>66 ± 13</td>
<td>68 ± 11</td>
<td>61 ± 13</td>
<td>67 ± 12</td>
</tr>
<tr>
<td>Female %</td>
<td>68</td>
<td>60</td>
<td>63</td>
<td>58</td>
</tr>
<tr>
<td>Digoxin 1st (ng/ml)</td>
<td>1.14 ± 0.77</td>
<td>1.38 ± 0.57</td>
<td>1.62 ± 0.74</td>
<td>1.12 ± 0.40</td>
</tr>
<tr>
<td>Digoxin 2nd (ng/ml)</td>
<td>1.19 ± 0.88</td>
<td>1.42 ± 0.60</td>
<td>1.74 ± 0.76</td>
<td>1.15 ± 0.48</td>
</tr>
<tr>
<td>Digoxin average (ng/ml)</td>
<td>1.15 ± 0.80</td>
<td>1.40 ± 0.55</td>
<td>1.68 ± 0.70</td>
<td>1.14 ± 0.43</td>
</tr>
<tr>
<td>Creatinine Clearance ≤ 50 ml/min</td>
<td>34</td>
<td>33</td>
<td>23</td>
<td>36</td>
</tr>
</tbody>
</table>

93% in group I, 80% in group II, 75% in group III and 84% in group IV had therapeutic digoxin levels. Low creatinine clearance, age over 65 and drug interactions were risk factors associated with supratherapeutic levels, mostly seen in group II and group III with 20% and 24% respectively.

Conclusions: 1. Digoxin plasma levels proportionally increase as the weekly dose increases 2. Similar therapeutic plasma levels are observed in group I, II and IV 3. High proportion of supratherapeutic levels are seen when Digoxin is used with Amiodarone and in patients with Creatinine clearence smaller than 50 mL/min.
The chronic myeloproliferative neoplasms (MPNs) are clonal disorders characterized by excess proliferation and normal maturation of cells from one or more myeloid lineages. The most common MPN is chronic myeloid leukemia (CML), polythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Rare subtypes include chronic eosinophilic leukaemia (CEL), chronic myelomonocytic leukaemia (CMML) and systemic mastocytosis (SM). The constitutive activation of the BCR-ABL protein tyrosine kinase as consequence of the reciprocal translocation t(9;22) in CML has become the paradigm for molecular pathogenesis and targeted treatment with tyrosine kinase inhibitors, e.g. imatinib. Meanwhile, it has become evident that acquired constitutive activation of protein tyrosine kinases is also a central feature in the pathogenesis of BCR-ABL-negative MPN. The most commonly involved genes are the receptor tyrosine kinases and the non-receptor tyrosine kinases JAK2 and ABL. Activation occurs as a consequence of specific point mutations or diverse fusion genes generated by chromosomal translocations, insertions or deletions. Mutant kinases are constitutively active in the absence of the natural ligands resulting in deregulation of hemopoiesis in a manner analogous to BCR-ABL in CML. The most common point mutation is JAK2 V617F which is identified in most patients with PV and about 50% of patients with ET and PMF while a Kit D816V mutation is found in 80-90% of patients with SM. The most common fusion gene is PDGFRα-PDGFRβ which is present in a substantial proportion of patients with CML, small molecule RTK high response rate to patients associated with constitutive activation of PDGFRα-PDGFRβ, ABL and some KIT mutants. Other inhibitors under development are promising candidates for effective treatment of patients with constitutive activation of JAK2, FGFR1 and imatinib-resistant KIT mutants. The indolent clinical course of distinct subtypes, e.g. PV or ET, and the efficacy and safety of currently used therapies necessitate that JAK2 inhibitors have a favourable toxicity profile in the short- and long-term. Important questions for future studies remain the unknown molecular pathogenesis of approximately 30% of MPN patients and the potential role of additional genetic events in the pathogenesis of MPN. 

Received Tyrosine Kinases As Therapeutic Targets In Malignant Glioma, Present Status And Application In China

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The chronic myeloproliferative neoplasms (MPNs) are clonal disorders characterized by excess proliferation and normal maturation of cells from one or more myeloid lineages. The most common MPN is chronic myeloid leukemia (CML), polythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Rare subtypes include chronic eosinophilic leukaemia (CEL), chronic myelomonocytic leukaemia (CMML) and systemic mastocytosis (SM). The constitutive activation of the BCR-ABL protein tyrosine kinase as consequence of the reciprocal translocation t(9;22) in CML has become the paradigm for molecular pathogenesis and targeted treatment with tyrosine kinase inhibitors, e.g. imatinib. Meanwhile, it has become evident that acquired constitutive activation of protein tyrosine kinases is also a central feature in the pathogenesis of BCR-ABL-negative MPN. The most commonly involved genes are the receptor tyrosine kinases and the non-receptor tyrosine kinases JAK2 and ABL. Activation occurs as a consequence of specific point mutations or diverse fusion genes generated by chromosomal translocations, insertions or deletions. Mutant kinases are constitutively active in the absence of the natural ligands resulting in deregulation of hemopoiesis in a manner analogous to BCR-ABL in CML. The most common point mutation is JAK2 V617F which is identified in most patients with PV and about 50% of patients with ET and PMF while a Kit D816V mutation is found in 80-90% of patients with SM. The most common fusion gene is PDGFRα-PDGFRβ which is present in a substantial proportion of patients with CML, small molecule RTK high response rate to patients associated with constitutive activation of PDGFRα-PDGFRβ, ABL and some KIT mutants. Other inhibitors under development are promising candidates for effective treatment of patients with constitutive activation of JAK2, FGFR1 and imatinib-resistant KIT mutants. The indolent clinical course of distinct subtypes, e.g. PV or ET, and the efficacy and safety of currently used therapies necessitate that JAK2 inhibitors have a favourable toxicity profile in the short- and long-term. Important questions for future studies remain the unknown molecular pathogenesis of approximately 30% of MPN patients and the potential role of additional genetic events in the pathogenesis of MPN. 

Background: Receptor tyrosine kinases (RTK) and related cellular signaling pathways are important targets for treatment of malignancies. As novel anti-cancer agents, small molecule RTK inhibitors are increasingly being used in patients with constitutive activation of RTK and the non-receptor tyrosine kinases JAK2 and ABL. In addition, we briefly review application of some of the RTK inhibitors in the same malignancy.

Methods: Key inhibitors of genes were analyzed by qRT-PCR and western blotting in malignant glioma cells. Proliferation was measured by MTT assays and drug synergy was assessed by Chou-Talalay method; Cell cycle and apoptosis blotting in malignant glioma cells. Proliferation was measured by MTT assays and drug synergy was assessed by Chou-Talalay method; Cell cycle and apoptosis were analyzed by flow cytometry and migration by monolayer migration assays. siRNA was applied to knockdown platelet-derived growth factor receptor-B (PDGFRB) expression in glioma cells. Multi-immunoblot was performed on Imatinib-treated and control cells.

Results: Imatinib treatment was lack of growth inhibition activity in glioma cells. The treatment was more effective in inhibiting the phosphorylation of Bcr-Abl in K562 cells than that of PDGFR in glioma cells. Knockdown of PDGFRB by siRNA showed similar responses on an Antibody microarray as in the case to imatinib treatment in U87 glioma cells. In addition, imatinib induced specific changes in downstream signalling pathways of the cells. Most prominently, constitutive activation of extracellular signal-regulated kinase (ERK1/2) signalling and its downstream effectors, e.g. dual phosphatases and immediate early genes (IEG), MAPK and -3, and ribosomal protein S6 (rPS6) were strikingly increased or altered in both imatinib and qRT-PCR analysis. Continuous activation of ERK1/2 induced by imatinib treatment was related to S-phase re-entry in U87 cells.

Conclusions: In conclusion, a complex and dynamic modulation of downstream signal transduction pathways in glioma cells; 2) Synergistic drug synergy was assessed by Chou-Talalay method; Cell cycle and apoptosis were analyzed by flow cytometry and migration by monolayer migration assays. siRNA was applied to knockdown platelet-derived growth factor receptor-B (PDGFRB) expression in glioma cells. Multi-immunoblot was performed on Imatinib-treated and control cells.

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Conclusions: In conclusion, a complex and dynamic modulation of downstream signal transduction pathways in glioma cells; 2) Synergistic drug synergy was assessed by Chou-Talalay method; Cell cycle and apoptosis were analyzed by flow cytometry and migration by monolayer migration assays. siRNA was applied to knockdown platelet-derived growth factor receptor-B (PDGFRB) expression in glioma cells. Multi-immunoblot was performed on Imatinib-treated and control cells.
Neonatal Abstinence Syndrome And Cerebral Infarction Following Maternal Codeine Use During Pregnancy

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Background: Neonatal withdrawal from maternal drugs is common in many neonatal intensive care units. Codeine-containing cough preparations given to pregnant mothers can cause neonatal abstinence syndrome. Many women do not consider prescription cough syrups when asked about drug use. Maternal medication or illicit drug use, including opiates, has been identified as a cause of perinatal arterial stroke. Since codeine is an opiate with pharmacodynamic effects similar to morphine, it is reasonable to investigate if maternal codeine use can have effects on the fetus similar to other opiates, including cerebral infarction.

Methods: We present 3 cases of newborn infants with perinatal arterial stroke associated with in utero exposure to codeine. The first infant had infarction of the left middle and anterior cerebral arteries. Her mother received a codeine-containing cough medicine for an URI for 2 weeks prior to delivery. The second infant had infarction of the left occipito-temporal region. The mother had been hospitalized with pneumonia and received a codeine-containing cough medicine also for about 2 weeks prior to delivery. A third case has recently been added. None of the infants had other identifiable causes for cerebral infarction. All presented with seizures and signs of neonatal abstinence. All were eventually discharged to home.

Results: Perinatal arterial stroke can be a consequence of many illegal drugs, including opiates. Neonatal abstinence syndrome as a result of maternal codeine use has been discussed, even in irradiated mothers. Opiates have been shown to cause neonatal thrombocytopenia, apoptosis in microglia and neurons and vasoconstriction in the placental circulation and middle cerebral arteries. It is reasonable to assume that codeine, may have similar effects on the fetus when administered to the pregnant mother.

Conclusions: Physicians should investigate maternal medication use, including codeine-containing cough preparations, when evaluating newborn infants with neonatal abstinence syndrome and/or evidence of cerebral infarction, particularly if the mother does not have a history of illegal drug use. This discussion raises questions about the safety of codeine-containing treatments and other “magic bullets” for pregnant women.

Methicillin Resistance In Staphylococcus Aureus And Coagulase-Negative Staphylococci

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Background: Staphylococcus species are divided into coagulase-negative staphylococci, represented by S. aureus and coagulase-negative staphylococci (CNs). Oxacillin has been one of the main drugs used for the treatment of staphylococcal infections; however, a large number of S. aureus and CNs isolates of resistance were selected in placental circulations. Methicillin resistance is encoded by the mecA gene which is inserted in the SCCmec cassette.

Aims: 1) To detect the resistance to meticillin in S. aureus and CNS isolates from the Clinical Hospital of Botucatu Medical School, UNESP, Brazil. 2) To determine the minimum inhibitory concentration (MIC) to erythromycin, netilmicin, sulfamethoxazole-trimethoprim and vancomycin in S. aureus and CNS strains by the E-test technique.

Methods: A total of 150 samples were analyzed regarding methicillin resistance by detection of the mecA gene, agar diffusion technique using cefoxitin and oxacillin disks, screening test on Mueller-Hinton agar supplemented with oxacillin 6 mg/mL and 4% sodium chloride and E-test.

Results: A total of 102 isolates were identified as S. aureus and 48 as CNS. A total of 46 (45.1%) S. aureus and 37 CNS (81.2%) were mecA-positive. S. epidermidis was the most frequently isolated CNS species corresponding to 87.5% of all CNS strains investigated and 83.3% were mecA-positive. S. aureus and CNS also were tested in relation to erythromycin, with 56 (64.9%) and 31 (64.6%) isolates resistant to this drug, netilmicyn, with 42 (41.2%) and 30 (62.5%) isolates resistant, trimethoprim-sulfamethoxazole with 46 (45.1%) and 35 (72.9%) isolates resistant, respectively. All the isolates of S. aureus were sensitive to vancomycin and 12 (25%) of CNS were vancomycin-intermediate.

Conclusions: 1) Most of the strains of S. aureus and CNS was mecA-positive. 2) S. epidermidis was the species with a higher percentage of resistance to meticillin. 3) Among phenotypic methods, E-test yielded the best results in the detection of methicillin resistance. 4) The distribution of resistance to drugs showed difference between methicillin-resistant Staphylococcus and methicillin-sensitive isolates, with higher percentages of resistance to erythromycin, netilmicyn and trimethoprim-sulfamethoxazole in methicillin-resistant strains.

Financial support: FAPESP and FUNDEESP

Vindesine And Razoxane, An Effective Drug Combination For Soft Tissue Sarcomas

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Background: Vindesine and Razoxane were shown to enhance the radiation response and to suppress distan metastasis in animal systems. Both drugs affect main steps of the metastatic cascade. During the past 15 years, these drugs were evaluated in translational research studies in a variety of soft tissue sarcomas [STS] with emphasis given to the radiation response and the dynamics of metastasis.

Methods: In a phase II study, 23 patients with unresectable (n=7) and oligometastatic STS (n=16) received a basic treatment with razoxane (2x125 mg daily) and vindesine (2 mg /l/ week) supported by radiotherapy and occasionally by surgery. Long-term treatment was intended in patients with metastatic disease. Thirty-six patients with comparable stages and prognostic parameters treated with contemporary chemotherapy served as non-randomised, retrospective controls.

In addition, the outcome of a small multicenter study on vascular soft tissue sarcomas is reported. This study was performed by the Austrian Society of Radio-Oncology, using the same treatment regimen and study endpoints.

Results: In the first study, the combination of razoxane-vindesine-radiotherapy led to 89% major responses (CR + PR). The median number of new metastases after 6 months was 0 (range, 0-40) and after 9 months likewise 0 (0-70). The corresponding numbers in the control group were 4.5 (range, 0-40) and 9 (0-100). The progression-free survival at 6 months was 74% in the treated group up and 23% in the controls. The median survival from the occurrence of the first metastasis or time of unresectability was 20+ months versus 9 months [p=0.001]. The combined treatment was well tolerated but normal tissue reactions were enhanced.

In the OEGRO study, 6 of 8 patients with unresectable measurable angiosarcomas showed a CR. The median survival time from the start of the treatment is 23+ months for 12 patients with macro-scopic and microscopic residual disease. The progression-free survival at 6 months was 75%.

Conclusion: This trimodal treatment leads to excellent response rates at irradiated soft tissue sarcomas, it suppresses distant metastases and prolongs survival.
Pharmacokinetics (PK) And Pharmacodynamics (PD) Of Fosfomycin (FOF)
For Central Nervous System (CNS) Infections

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Background: The emergence of infections caused by multiresistant bacteria calls for new therapies. FOF could be used as part of a combined therapy for CNS infections since it shows favourable PK properties such as low molecular weight, negligible protein binding and good penetration into the uninflamed and inflamed cerebrospinal fluid (CSF). On the other hand, PD data of FOF in the CNS are scarce.

Methods: We conducted a selective literature research to compile in vivo PKPD data of FOF in CNS infections.

Results: Few reports have described PD data of FOF in CNS infections in humans and in animal models. FOF peak CSF levels, half-life (t½) and penetration into CSF are shown in the table. In the rabbit meningitis model, a bactericidal biomarker of intake was used to monitor effect of FOF intake on prognosis in samples.

This is due to the accuracy and reliability of LC-MS as an analytical technique and scarce protein binding and good penetration into the uninflamed and inflamed CSF are shown in the table. In the rabbit meningitis model, a bactericidal biomarker of intake was used to monitor effect of FOF intake on prognosis in samples.

Asparaginase Synthetase Inhibitors With Nanomolar Potency: An Unexplored Approach To Treating Drug-Resistant Leukemia

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Background: Clinical studies have identified an intriguing inverse correlation between the levels of asparagine in the blood and the susceptibility of leukemia cells to chemotherapy. Leukemia cells seem to become drug-resistant by over-expressing asparaginase synthetase (ASNS), an enzyme that catalyzes the intracellular synthesis of asparagine. Compounds that inhibit human ASNS represent potential drugs for treating acute lymphoblastic leukemia (ALL).

Aims: 1) To examine the effects of a potent ASNS inhibitor on the growth of a MOLT-4 cell line. 2) To develop an atomistic understanding of interactions formed between the inhibitor and ASNS.

Methods: The ASNS inhibitor was incubated at 0.1-1.0 M concentration with asparaginase-resistant MOLT-4 cells, which exhibit unregulated levels of ASNS expression. Cell viability was determined after 48 h using a WST-1 proliferation assay. The mean cell titer of treated samples was calculated, as the mean ± SD of triplicate experiments, relative to control cells. A model of the ASNS/inhibitor complex was built by homology to the structure of Esherichia coli AS-B, with the inhibitor being placed in the synthetase active site using flexible docking and molecular dynamics methods. Site-specific mutants of Glu-348 in Esherichia coli AS-B were expressed and purified by standard methods, and assayed using both steady-state kinetics and NMR-based 2H-exchange experiments.

Results: In the presence of L-asparagine, a spectrophotometric assay showed a decrease in the production of L-asparagine by MOLT-4 cells. The mean cell titer of treated samples was calculated, as the mean ± SD of triplicate experiments, relative to control cells. A model of the ASNS/inhibitor complex was built by homology to the structure of Esherichia coli AS-B, with the inhibitor being placed in the synthetase active site using flexible docking and molecular dynamics methods. Site-specific mutants of Glu-348 in Esherichia coli AS-B were expressed and purified by standard methods, and assayed using both steady-state kinetics and NMR-based 2H-exchange experiments.

Conclusions: 1) This study is the first direct demonstration that ASNS inhibitors can suppress proliferation of a drug-resistant MOLT-4 cell line in a dose-dependent manner. 2) The ASNS/inhibitor model provides a firm basis for discovering novel compounds with improved activity against ALL.


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Background: Medical pharmacology is based on receptor actions, using specific agonist and/or antagonist. The famous concept of Ehrlich:«corpora non agunt nisi quae ad eam agonist et antagonist aequatur» can be considered as a founding principle of receptor theory. No drug, no magic bullet can act without being recognized by a specific molecule which mediates either its penetration in the cell or the transfer of a specific «message» to the cell interior. Without this concept no specific drug action could have been developed. Ehrlich’s concept developed in the frame of his immunological experimentation, «the side-chain» concept was progressively extended to nearly all drug-cell interaction. One of the consequences of this conceptual advance was the recent increase of life expectancy, below 50 years at the turn of the 19th to the 20th century to above 80 years recently.

Experimental basis: This progress was based on the application of Ehrlich’s original concept to the pharmacology of age-related diseases, cardiovascular and respiratory pathologies. More recently a number of receptors were shown to decrease with age and some others «uncoupled» in aging cells from their normal intracellular transduction pathway with loss of its beneficial effects (Robert L, J. Gerontol. 1998;44-307-317). An example is the elastin receptor recognizing elastin sequences and triggering a calcium transient followed by modifications of several cell functions, chemotactic movements, NO release and vasoconstriction but also increase of elastase and free radical release. In old cells the calcium transient is damped, Ca increase producing apoptotic cell death (Robert L, Labat-Robert J. Biogerontology, 2000;1:123-131). The coupling of the elastin receptor to cGMP is interrupted (Faury et al, Mech. Age. Dev., 1997;95:31-42). We could show that these alterations play a critical role in the age-dependent decline of the cardiovascular and respiratory functions (Robert et al, Biogerontology. DOI:10.1007/s10522-007-9126-b).

Conclusions: The original receptor concept of Ehrlich could be applied to aging biology and to its crucial components the mediation and modulation of the interactions of cells with macromolecules of the extracellular matrix. With this enlargement, Ehrlich’s concept could be applied to aging biology and pharmacology.
Piperacillin Dosing In ICU Patients – New Magic For Old Bullets

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Background: The poor outcomes for intensive care unit (ICU) patients with sepsis require higher level research to be conducted to ensure antibiotic therapy is optimized. Altered dosing strategies may be the key to further improving patient outcomes. Aims: 1) To identify and analyze relevant pharmacokinetic studies of piperacillin in ICU patients. 2) To discuss pharmacodynamic optimization of piperacillin in ICU patients using 'non-standard' dosing strategies.

Methods: Relevant articles were identified from searches of PubMed and the extensive files of the authors including submitted and accepted original research articles not PubMed listed. Pharmacokinetic parameters between different patient populations were identified and compared. The effect of different dosing strategies on achieving pharmacodynamic endpoints (free piperacillin concentration maintained above the minimum inhibitory concentration of bacteria for at least 50% of dosing schedule; 50% T>MIC) was evaluated.

Results: Piperacillin has been investigated in various different patient populations with differences noted between various pharmacokinetic parameters, particularly clearance and volume of distribution. Piperacillin has non-linear clearance, although this becomes effectively linear at the doses used clinically in ICU patients. ICU patients have a higher volume of distribution and clearance than other patient populations, which results in bolus dosing producing a reduced capacity to achieve 50% T>MIC. In contrast administration by continuous infusion enables superior achievement of pharmacodynamic endpoints than bolus dosing (4mg six-hourly bolus, 58%, vs continuous infusion 16mgm/day, 94%).

Conclusions: 1) Various studies were identified showing altered piperacillin clearance and volume of distribution in various patient populations. 2) Dosing by extended infusion or continuous infusion achieves superior pharmacodynamic outcomes, which supports data on improved outcomes observed in previous retrospective clinical studies.

Enhancing Radiotherapy Of Cancer Using Agents That Target Thrombospondin-1 Signaling Via CD47

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Background: Radiation is a primary mode of cancer therapy that acutely damages cellular macromolecules and elicits stress responses leading to cell death. The known cytotoxic protective of nitric oxide is blocked by thrombospondin-1 (TSP1), a potent antagonist of nitric oxide/GMP signaling, suggesting that TSP1 signaling via its receptor CD47 could correspondingly increase radiosensitivity and that antagonists of this pathway could be effective radio protectants.

Methods: Wild type, TSP1-, TSP2- and CD47-null mice were subjected to 25 Gy hindlimb irradiation and studied over 8 weeks. Vascular cells isolated from the respective mice were irradiated in vitro at 10 to 40 Gy and analyzed for survival and proliferation. Radioprotective activities of antagonists were studied in vitro in vascular cells and in vivo using wild type mice.

Results: Twelve hours after 25 Gy hindlimb irradiation, TSP1 null mice showed significantly less cell death in muscle and bone marrow. Two months following irradiation, skin and muscle units in the null mice showed minimal histological evidence of radiation injury and near full retention of mitochondrial function. Tissue perfusion and acute vascular responses to NO are also preserved in irradiated TSP1 null hindlimbs. The role of TSP1 in radiosensitization is specific in that TSP2 null mice were not protected. However, mice lacking the TSP1 receptor CD47 showed similar radiosensitization as TSP1 null mice. TSP1- and CD47-dependent radiosensitization is cell autonomous because vascular cells isolated from the respective null mice showed dramatically increased survival and improved proliferative capacity following irradiation in vitro. Antisense suppression of CD47 expression effectively protected endothelial cells in vitro from radiation-induced death and preserved skin and muscle integrity and function in irradiated hindlimbs of wild type mice.

Conclusions: Soft tissues in TSP1 and CD47 null mice are remarkably resistant to radiation injury. This is due to a cell-autonomous radiosensitizing signal arising from TSP1 binding to its receptor CD47. Antagonists of this pathway can protect soft tissues from the deleterious effects of irradiation and thereby enable use of increased radiation doses for cancer therapy.
Background: The purine nucleoside adenine (ADO) regulates multiple cell functions. Its effects are mediated by at least four kinds of P1 purinergic receptors (A1, A2A, A2B, and A3). Adenine kinase (AK) is a key intracellular enzyme regulating intra- and extracellular ADO concentrations. Pharmacological targeting of AK can enhance ADO tissue levels. In this study, we examined the pharmacokinetics of a new adenine kinase inhibitor, the 4-N-(3′-N,N-dimethylamino)-6,7-dimethoxyquinazoline (DMAQ) and its effects on ADO concentrations in rat tissues.

Methods: Male Wistar rats (n=6 of each experimental group, body weight (BWT): 200-250g) were orally dosed with 30 and 90 mg/Kg BWT of DMAQ or vehicle (Control group – n=6). Tissue samples were collected 1h after treatment and immediately ground to a powder under liquid nitrogen. ADO extraction was performed from tissues, derived with 2-chloroacetaldehyde and analysed by high performance liquid chromatography method (HPLC). To pharmacokinetic studies, blood was sampled in tubes containing heparin at control, 15, 30, 60, 120, 180, 240, 360, 420 and 480 minutes. Samples were collected from a catheter previously implanted into the femoral artery.

Results: Oral administration of DMAQ (30 mg/Kg) was demonstrated to increase ADO concentration in rat tissues such as heart (43.0±5.5%), liver (33.4±1.8%) and brain (24.0±4.9%). Pharmacokinetic parameters were determined for orally administered DMAQ (80 mg/Kg). The DMAXI maximal concentration in plasma of 22.90 µmol L−1 was reached at 3h after administration and after 7h, DMAQ concentration approached the limit of HPLC quantification. DMAQ showed a rapid absorption profile with an estimated absorption half-life of 13 min, and a moderately fast rate of elimination (93 min). Values for AUC0-24h, and volume of distribution were 180.08 µmol L−1 min−1 and 20.09 L/kg.

Conclusions: 1) DMAQ rapidly and consistently increased ADO levels in rat tissues. 2) DMAQ was shown to have favorable pharmacokinetics, indicated by a rapid absorption after orally dosed, wide distribution and moderate rate of excretion. Therefore, DMAQ may be considered as a good alternative for systemic inhibition of AK that can potentially be used for the treatment of cardiovascular and neurological diseases.

Authors' disclosure statement: DMAQ concentration in plasma and ADO tissue concentration were measured by high performance liquid chromatography methods.

Systemic Immunosuppressive Therapy With Oral Sirolimus After Bare Metal Stent Implantation: The Missing Alternative To Prevent Restenosis Following Percutaneous Coronary Interventions

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Background: In previous randomized study, ORAR III (Oral Rapamycin in Argentina), we demonstrated a significant reduction of restenosis with oral rapamycin under stent implantation (BMS) implantation. Its role in competition with drug eluting stents (DES).

Results: Since January 2006 to September 2007 at the Cardiac facilities of three Hospitals in Buenos Aires, Argentina, 200 patients with de novo coronary lesions were randomized to treated with OR plus BMS (100 pts) or with DES (100 pts). OR was given as a bolus of 10 mg per kg of body weight before PCI and dosed by daily doses of 3 mg per os for 12 days after PCI. Primary End points were to compare Hospital, Follow Up and Overall cost per patient at one, two, three and five years of follow up. Safety endpoint is a death from any cause, Q and non Q myocardial infarction (MI) and stroke was analyzed as Major Adverse Cardiovascular Events. Target Vessel (TVR) and Target Lesion Revascularization (TLR) were independently analyzed as End point. Direct cost in US dollars included procedural resources, hospitalization, medications, repeat revascularization procedures and professional.

Results: Baseline demographic, clinical and angiographic characteristics were similar in both groups. At 12 ± 1 months of follow up, the rates of clinically driven TLR and TMR were similar with both strategies: TVR per vessel was 8.4% with OR versus 7.7% with DES, TLR per lesion was 5.7% with OR versus 5.8% with DES. Cumulative probability of MACCE was not significantly different although during follow up patients treated with DES had significant high incidence of MACCE compared to OR group (13% vs 3% p=0.001) Initial and cumulative costs were in favour of DES treated patients (p=0.0001).

Conclusions: At 12 months of follow up and initial strategy with OR plus BMS is cost saving and effective compared to DES in patients with de novo lesions undergoing PCI.

Cytochrome P450 Dependent Drug Response In Oncology

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Cytochromes P450 (CYP) in families 1-3 are responsible for most of the phase I hepatic biotransformations of drugs. The polymorphic nature of these enzymes causes large inter-individual differences in drug disposition that can result in clinical drug toxicity. This polymorphism, in this way, specific genotypes within patients could be used to individualize drug therapy. In addition to hepatic metabolism, drug efficacy can be modified by the target tissue metabolism. For example, an altered drug metabolizing capacity in cancer cells could result in drug resistance to anticancer drugs. Here we illustrate these different situations with two clinically performed studies and present our novel findings.

Concerning the relevance of CYP variants for drug toxicities, we investigated the association between paclitaxel metabolism and CYP2C8 polymorphisms mediated with paclitaxel metabolism. Paclitaxel is an effective anti-cancer drug widely used, but its neurotoxicity is one of its most important side effects. Paclitaxel neurotoxicity has a large inter-individual variability of unknown molecular basis, and it represents a major challenge for the improvement of paclitaxel therapy. Since paclitaxel pharmacokinetic parameters have been shown to be related to neurotoxicity, we selected polymorphisms in the CYP2C8 gene. This analysis revealed a significant association between CYP2C8 gene and paclitaxel neurotoxicity.

Concerning the efficacy of anti-cancer drugs and the genetic background of the cancer cells, we showed that the expression of CYP3A4 in peripheral T-cell lymphoma (PTCL) tumors was inversely associated to the survival of the patients. PTCLs are clinically aggressive tumors typically showing less than 30% 5-years overall survival. To date, the most effective therapy is a combination chemotherapy regimen, in many cases including CYP3A4 (cytostaphamide, doxorubicin, vincristine and prednisone), but the poor clinical outcome of most patients clearly reveals the need for new factors affecting the response. Since the CYP3A4 subfamily enzymes are involved in the activation of chemotherapy drugs, we hypothesized that CYP3A4 expression in these lymphomas could result in a poor clinical response. We measured tumoral CYP3A4 and MDR1 hRBA content in 44 T-cell lymphomas finding a large variation in the expression. A high tumoral CYP3A4 expression was significantly associated with a poor drug response and survival of the patients. In conclusion, drug efficacy and toxicity can be altered by genetic variations in CYPs, in the case of chemotherapy toxicity while, in the case of efficacy, alterations in the target cells can also be critical.
Isoproterenol And Insulin: The Ying-Yang Of Regulation Of Mg2+ Homeostasis In Mammalian Cells

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Background: The investigation of the mechanisms regulating cellular Mg2+ homeostasis has highlighted a novel effect of isoproterenol and insulin on mammalian cells.

Methods: Over the years, studies were conducted in whole anesthetized animals, perfused organs, isolated cells as well as purified subcellular organelles from normal or diabetic animals.

Results: Isoproterenol administration results in the rapid extrusion of a sizable amount of cellular Mg2+ via a putative Na+/Mg2+ exchanger. This extrusion primarily mobilizes Mg2+ from cytoplasm and mitochondria. In contrast, treatment with insulin abolishes isoproterenol-induced Mg2+ extrusion and elicits per se an accumulation of Mg2+ from the extra-cellular compartment into the cell, which in certain cell types (e.g. cardiac myocytes) is associated with glucose accumulation. Further evidence of this opposite regulation is provided by diabetic conditions. The reduced or absent responsiveness to insulin, in fact, results in a marked decrease of Mg2+-within several tissues including skeletal muscles, heart, and liver, and in an enhanced responsiveness to isoproterenol or catecholamine stimulation, which further decreases tissue Mg2+ level and impairs the operation of several Mg2+-regulated enzymes.

Conclusions: These results provide a compelling evidence for the role of isoproterenol and insulin in regulating Mg2+ content within mammalian tissues. Together with results provided by other laboratories they highlight a key role of Mg2+ in regulating various cellular functions under physiological conditions.

Computed Tomographic Study Of The Paranatal Sinuses And Nasal Washings In Atopic Children Without Sinusitis Symptoms

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Background: The aim of this study is to correlate the extent of sinus disease with eosinophilia and inflammatory cells in nasal fluid (NF).

Methods: We studied 48 atopic children with allergic rhinitis (33 asthmatics); 15 non-atopic children served as a control group. Neither patients nor controls had sinusitis symptoms. Coronal computed tomographies (CT) were graded following a standard protocol. A sinus CT score >12 indicated severe disease. Nasal wash cytology (NW) expressed as a number of cells/mL of volume recovered, was followed by differential counts.

Results: 36/48 patients (19%) had extensive disease, 7 were asthmatics and 2 had only rhinitis; 39/48 and 13 controls had a CT score <12. Atopics had significant eosinophilia in NF and peripheral blood (Table). Total cell counts in NL were higher in atopics than in the control group. The differential cell counts in NW were similar in atopics regardless the CT score. There was correlation between CT score, peripheral eosinophil and NL eosinophil counts in atopics.

<table>
<thead>
<tr>
<th>Group</th>
<th>CT score</th>
<th>n</th>
<th>Blood eosinophil</th>
<th>Cells/mL</th>
<th>Eosinophils</th>
<th>Neutrophils</th>
<th>Epithelial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic</td>
<td>&gt;12</td>
<td>9</td>
<td>665</td>
<td>1305</td>
<td>757</td>
<td>137</td>
<td>137</td>
</tr>
<tr>
<td>Control</td>
<td>&gt;12</td>
<td>13</td>
<td>558</td>
<td>578</td>
<td>208</td>
<td>86</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>&lt;12</td>
<td>13</td>
<td>148</td>
<td>390</td>
<td>0</td>
<td>167</td>
<td>113</td>
</tr>
</tbody>
</table>

*Mean ±SD; Nasal Fluid Cell counts x 10^3/mL.

Conclusions: Extensive sinus disease is frequent in atopics without sinusitis symptoms (19%). Cytology of NF did not identify these patients among atopics. Eosinophils may be involved in extensive sinus mucosa inflammatory changes.
Backgroud: Previously, we showed that combination therapy with natural compounds such as curcumin and epigallocatechin gallate significantly suppressed estrogen receptor (ER)-negative tumor growth in vivo. Therefore, we synthesized 8 novel compounds with the aim to increase efficacy and bioavailability. These compounds were screened for their ability to elicit cytotoxicity, apoptosis and alter cell cycle progression in ER-negative breast cancer cells. The most potent compounds were also examined for their oral bioavailability.

Methods: MDA-MB-231 and SkBr3 human breast cancer cells were incubated with new chemical entities (0 – 25 µM) for 5 days and cell number was determined using the sulforhodamine B assay. Cell cycle distribution was assessed using propidium iodide staining. The presence of apoptosis was assessed using Annexin-V-FLUIOS/propidium iodide staining. The proportion of cells in each of G_0/G_1, S- and G_2/M-phases as well as the proportion of apoptotic cells (as a percent of total cells) was determined using a flow cytometer. All data are expressed as the mean ± SEM from 4 independent determinations performed in triplicate. Statistical significance was determined using an ANOVA and a Student Newman-Keuls post hoc test, where p<0.05 was required for a statistically significant difference.

Results: Three compounds (RL75, RL84 and RL86) were less potent than curcumin in both in vitro and in vivo assays. The highest cytotoxic potential in both MDA-MB-231 and SkBr3 cells with IC_50 of ~1 µM and 0.5 µM, respectively. RL91 (2 µM) induced an increased proportion of cells in G_0/G_1 phase as well as the proportion of apoptotic cells (as a percent of total cells) was determined using a flow cytometer. All data are expressed as the mean ± SEM from 4 independent determinations performed in triplicate. Statistical significance was determined using an ANOVA and a Student Newman-Keuls post hoc test, where p<0.05 was required for a statistically significant difference.

Conclusions: 1) RL90, RL91 and RL92 elicited the greatest cytotoxicity in both ER-negative cell lines. 2) RL91 elicited the greatest apoptotic response. 3) RL92 elicited the greatest degree of G_1 arrest, mirroring its increase in G_1 phase cells and was only readily observed in female mice. 4) Currently, RL92 is our best drug candidate and is undergoing further testing in mouse models of ER-negative breast cancer.

Abstract: Cannabis sp. is a ubiquitous dioecious plant genus that grows wild in most countries. This plant, and its 66 pharmacologically active cannabinoids (CBs), has been used for industrial purposes for at least 10,000 years and medically for more than 2,700 years. Historically, wars have focused upon the need for industrial hemp by seafaring nations. For example, Napoleons attempted to block passage of hemp from Russia to England in 1807. Because of the legal and political climate, CBs are not utilized in treating cancer related symptoms such as nausea and anorexia. Current research reveals that cannabis and other mammals, have an intimate relationship with CBs as an endogenous CB system distributed throughout most organs of the body. Two endogenous CBs, anandamide and 2-arachidonoylglycerol (2-AG), and exogenous CBs have been implicated in a number of disease processes including Alzheimer’s disease, amyotrophic lateral sclerosis, Tourette’s syndrome, diabetes, dystonia, fibromyalgia, gastrointestinal disease, glaucoma, hepatitis C, human immunodeficiency virus related symptoms, hypertension, incontinence, multiple sclerosis, osteoporosis, rheumatoid arthritis and sleep apnea, via the CB receptors CB1 and CB2. More recently, CB receptors have been shown to interact with a number of neurotransmitters, such as the opiate and glutamate systems. Generally, CBs dampen the excitatory effects of other excitatory neurotransmitters in the central nervous system; hence, the development of the novel synthetic CB for appetite control, e.g., rimonabant.

Conclusions: Once CBs and the potential hypotherbole regarding the pros and cons of medical marijuana, research may indeed reveal that the CB system is one of Professor Erlich’s “magic bullets.”
Genetic Polymorphisms Of Cytochromes P450 Influence The Leflunomide Treatment And Toxicity In Rheumatoid Arthritis Patients

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Background: Leflunomide is a disease-modifying antirheumatic drug that is used for the treatment of rheumatoid arthritis (RA). Upon oral absorption, it is converted to the active metabolite (A77 1726) that inhibits de novo synthesis of pyrimidine ribonucleotides. In vitro studies have demonstrated that cytochromes P450 (CYPs), mainly CYP1A2 and CYP2C19 may be involved in leflunomide activation. The hypothesis of our study was that genetic polymorphisms of CYP1A2 and CYP2C19 influence the A77 1726 serum concentrations and the response of leflunomide treatment in RA patients.

Methods: A genotyping approach was used to determine CYP1A2 -163C>A, CYP2C19*2 and CYP2C19*17 genotypes in 112 RA patients. Trough steady-state A77 1726 serum concentrations were determined by validated HPLC with UV detection in all patients on leflunomide treatment.

Results: The leflunomide treatment was well tolerated by 62 patients, while 50 patients discontinued the treatment within the first year due to ineffectiveness (N=7), toxicity (N=37) or both (N=6). CYP2C19 genotypes did not influence the treatment response (P= 0.258), while an association between CYP1A2 C-163A polymorphism and leflunomide toxicity was observed. Patients with the CYP1A2 -163A allele (homozygous or heterozygous) had 11.8-fold higher risk for leflunomide-induced toxicity as compared to carriers of the CYP1A2 -163A allele (P= 0.001, OR: 11.93, 95% CI: 2.793-50.980). On the other hand, mean values of A77 1726 serum concentrations were not significantly different between CYP1A2 -163CC and CYP1A2 -163CA+AA genotypes (43.9± 39.5 mg/L and 33.7± 28.9 mg/L, respectively). Nevertheless, carriers of the CYP2C19*2 allele had significantly lower mean values of A77 1726 serum concentrations as compared to patients with CYP2C19*1/*1 genotype (18.4± 12.8 mg/L vs. 43.7± 33.7 mg/L, P= 0.005).

Conclusion: Our results suggest that the CYP1A2 -163C>A polymorphism influenced the leflunomide toxicity, while genetic polymorphisms of CYP2C19 had an impact on the A77 1726 serum concentrations in RA patients.

Some New Derivatives Of Vindoline, Monoindole Catharanthus Alkaloid

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Vindoline and catharanthine, monoindole alkaloids from Catharanthus roseus (L.) G. Don, are valuable precursors of oncolytic drugs, vinblastine and vincristine, used in treatment for some types of cancer. Due to the availability of Polonosky-Potier and some kinds of oxidation reactions resulting in coupling of monoindole alkaloids into bisindole ones, the search for semi-synthetic analogues of natural precursors is still of interest. In this communication five new vindoline derivatives obtained by means of Suzuki-Miyaura reaction, some new (hetero)dimers and other vindoline oxidation products as well as results of their preliminary cytotoxicity tests are reported.

Dissemination And Communication: “Selling” Vaccines To Peers And The General Public

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Background: In a society that relies on herd immunity as a health protection measure, informing peers and the general public about immunisation can be precarious without access and knowledge of effective communication strategies.

Aims: 1) To compare immunisation discourses on two vaccines: MMR and DTP. 2) To compare peer versus general public directed discourse. Focus include both descriptions of vaccines and the diseases they target. Special attention will be given to controversies.

Methods: Discourses analysed include: medical research articles in the British Medical Journal and the Lancet, British newspaper articles, and British websites maintained by the National Health Service or by private organisations. Discourses were coded manually. Cognitive Discourse Analysis was used and both qualitative and quantitative measures were obtained.

Results: Vaccine discourses were found to be very similar. The main differences lie in the target audience and in the purpose of the discourse. Medical research articles instigating controversies differ significantly in several respects (e.g. vague language, “name dropping” and high variability of titles for professionals) from typical ones. Patterns of “over-compensation” were found in both lay and professional discourses. The concept of herd immunity is very strikingly present even in professionals’ discourse aimed at the general public, whereas peer-to-peer discourse empowers the reader in the discourse.

Conclusions: 1) Proponents, opponents and reporters on vaccines employ typical ones. Patterns of “over-compensation” were found in both lay and professional discourses. The concept of herd immunity is very strikingly present even in professionals’ discourse aimed at the general public, whereas peer-to-peer discourse empowers the reader in the discourse.

Abstracts
Cationic Liposomes: How A Magic Bullet Turns Into An Anti-Inflammatory Agent

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Background: Cationic lipids are mainly used as efficient DNA, RNA or protein carriers for gene therapy or immunization trials. Significant progress has been made in the understanding of the cellular pathways and mechanisms involved in lipoplex-mediated gene transfection but the interaction of cationic lipids with cell components and the consequences of such an interaction on cell physiology remains poorly described.

Methods: RAW 264.7 macrophage cell line was cultured in DMEM (Invitrogen) supplemented by 5%FBS, 1 mM sodium pyruvate, 1 mM glutamine and antibiotics (Invitrogen). Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats (obtained from local routine blood donations) by density centrifugation.

Results: Interestingly, diC14-amidine liposomes stimulate, like LPS, myeloid dendritic cells through Toll-like receptor 4 activation. These findings suggest that cationic liposomes alone are potent immunostimulatory adjuvants that could stimulate efficiently the immune system and open the way to the use of cationic lipids as unique adjuvant components of vaccine. On the other hand, diC14-amidine liposomes inhibit CpG sequences or lipopolysaccharide-induced cytokine secretion by macrophages. This inhibitory effect was also mediated by the phospholipidic fraction of lipoproteins and synthetic phospholipids suggesting that cationic liposomes confer to these phospholipids new anti-inflammatory properties.

Conclusions: In summary, the examples reported in the present review highlight the dual nature of cationic liposomes: carriers and agents that modulate cellular responses (1). Most cationic lipids transport hydrophilic materials (DNA, RNA, proteins) into the cell but can also modify specific cellular activities (immunostimulatory and anti-inflammatory properties). Novel strategies, based on the co-existence of these two functions, have already been elaborated successfully. Combining the immunostimulatory activity of cationic liposomes with their carrier property could be a way to act simultaneously on the immune system and the target cell.

Hepatoprotective Activity of Herbal Extracts Used in Iranian Traditional Medicine against Acetaminophen-Induced Liver Injury in Mice

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Background: Strategic location of liver between intestinal tract and the rest of the body facilitates its task of maintaining metabolic homeostasis, secretary and excretory functions in the body. Therefore, the key organ in the body is the dominant target site of specific toxins. Accordingly, liver disorders are numerous and varied. Species of Scrophularia, Plantago psyllium and Fumaria officinalis showed the most resistance to the even new appropriate antibiotics, and some species were completely resistant to the tested antibiotics, including cefepime and tobramycin.

Methods: The samples were collected from impounded who had infections unresponsiveness to the routine classic treatments. After culturing and isolation of the bacteria, using disc diffusion and minimum inhibitory concentration (MIC), the sensitivity tests to the appropriate antibiotics for each sample was achieved.

Results: The type of bacteria were including; pneumococcus (9%), staphylococcus positive coagulase (4%) and negative coagulase (21.3%), pseudomonas aeruginosa (18.8%), klebsiella (25.6%), E.coli (26.8%), shigella (1%), proteus (0.2%) and acinetobacter (1%). Among these bacteria staphylococcus aureus, pseudomonas aeruginosa, klebsiella and acinetobacter showed the most resistance to the even new appropriate antibiotics, and some species were completely resistant to the tested antibiotics, including cefepime and tobramycin.

Conclusions: This study showed a high and dangerous nosocomial resistant of which no non-intensive sanitary programs and hospital disinfection to control nosocomial infection. Also the study should determine whether antimicrobial therapy is warranted for a patient and is one agent or combination of agents necessary to eradicate the infection with optimal dose, duration, and route of administration.

Under-treated painful condition may lead patients to opioid abuse

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Objective: To find the prevalence of Opioid abuse caused by a poorly treated painful disease.

Introduction (Background): Despite the physician reluctance to prescribe Opioids, the use of Opioid drugs for acute and chronic non-cancer pain is increasing. Although these drugs provide an important analgesic effect, they impose the risk of substance abuse to the addiction prone patients. The systematic literature reviews among the patients treated with Opioid drugs for acute or sub-acute painful conditions could not adequately answer whether the risk for iatrogenic addiction among these patients is relatively high. In order to get the incidence of Opioid abuse in a society in which the Opioid drugs are not easily available to treat painful conditions, this study was designed.

Design: By doing a pilot study the prevalence of Opioid- abuse following a poorly treated painful disease among the patients with the Opioid was determined and the size of the study participant was estimated. All the patients were evaluated via a psychiatric interview to determine the existence of Opioid abuse based on the criteria of Diagnostic and Statistical Manual of Mental Disorders (DSM IV). Also Minnesota Multiphasic Personality Inventory (MMPI) test was performed for all the patients. Provided they claimed a painful condition lead them to the Opioid abuse, a thorough physical examination was performed to find the somatic signs of the nociception.

Results: Twenty-six out of two hundred fifty participants had an organic source of pain.

Conclusion: Opioids are useful drugs in the treatment of acute and chronic non-cancer painful conditions. But inappropriate indications and problems with the overuse along with the problems of under prescribing due to the fear of addiction and regular dose control could urge the patients to Opioid abuse even criminal diversion.
Stenotrophomonas maltophilia Bloodstream Infections in Haematology and Non-Haematology Patients—A 5-year survey in Southwest Wales

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Background: Stenotrophomonas maltophilia is an aerobic, ubiquitious gram-negative bacillus which is of low pathogenicity and is rarely involved in causation of community-acquired infections. However it is increasingly becoming a hospital-acquired pathogen in immunocompromised patients. We report a 5-year survey from South Wales.

Methods: The study involved reviewing patient data from laboratory information management system (LIMS) and the National Public Health Laboratory (NPL) Datastore program using S. maltophilia as the key word.

Results: There were 1041 isolates of S. maltophilia from various clinical specimens from 865 patients. There were 99 episodes of bacteremia in 69 patients during the survey period. Bacteremias in 15 of the patients were regarded as contaminants on clinical grounds. Of the 54 patients with significant sepsis due to S. maltophilia, eighteen patients were suffering from haematological malignancies (9 had acute myeloid leukaemia, AML), while 16 were intensive care patients 4 of whom had burns, and 20 were general medical/surgical patients. The 30-day mortality rate for all patients was 46% (25/54) and in haematological patients it was 65% (11/17). The possible risk factors that were evident were immunosuppression especially in patients with AML and the use of broad-spectrum antibiotics especially the carbapenems where 63% (34/54) of all patients were recently on either mesnopenem or imipenem. In haematology-oncology patients 82% (14/17) of patients were on carbapenems. The susceptibility (to the antibiotics tested by BSAC methods) were as follows: amoxicillin (7%), co-amoxiclav (9%), aztreonam (26%), ciprofloxacin (11%), erythromycin (9%), gentamicin (16%), ciprofloxacin (87%), tigecycline (63%), timipenem (3%) and piperacillin/tazobactam (88%).

Conclusions: S. maltophilia can cause significant morbidity and mortality in hospitalized patients especially those who are immunocompromised or under intensive care and the appropriate antimicrobials should be considered when it is isolated. The excessive use of carbapenems and other antibiotics may be selected for S. maltophilia infections. Further research is urgently needed to develop antimicrobials that are active against S. maltophilia.

Polyclonal Rabbit Antiserum Against Porcine-SPA Able to Detect Human-SPA—A Using Immunological Methods

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Background: The lung collects SPA- and SP-D are present in a number of biological fluids and secretions what could make them disease markers. The bovine laevoglucosan (BAL) is a sample that permits to detect the proteins secreted by the lung epithelium, in a 1500-bed tertiary referral university teaching hospital; 2) to determine 30-day mortality attributable to S. maltophilia; 3) to select for S. maltophilia infections. Further research is urgently needed to develop antimicrobials that are active against S. maltophilia.

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Background: In the midst of rampant drug resistance, understanding the mechanisms of drug action can aid fast evolution of better drugs. Aim: To find roles of aromatics, helicity and valency in the action of de novo designed antimicrobial peptides.

METHODS: An amphipathic, cationic decapeptide Ac-GKRN’K’CKH’XVA-NN’H’ was designed and peptides with X= didehydrophenylalanine (dFm, aromatic,i-c) and aminoisobutyric acid (Um, aliphatic,i-d) were synthesized. Lysine branched bivalent dendrimers of all three (Us, Ud, Uf) and a non-helical diasteroisomer (nu-Fd) of Uf were also synthesized. Minimum Inhibitory (MIC) and Bactericidal (MBC) concentrations against E.coli and S.aureus, FACS, fluorescence and electron microscopy, RPHPLC and gel electrophoretic methods were used to study mode of action.

Results: Bacteriostatic effects were nil (Um), mild and transient (Ifm) and strong and persistent (i-Fm). Even though at par in binding to Lipopolysaccharide, i-Fm and Fm, but not Um, caused outer membrane permeabilisation. Inner membrane permeabilisation was attenuated and membrane architecture rehabilitated with i-Fm but not Fm, RPHPLC revealed that i-Fm was translocated into E.coli while Um and fragments of Fm were detected in the medium. Among monomers, only i-Fm was modestly antibiotic (MICs 110 µM (E.coli), 450 µM (S.aureus)). However, its dendrimer i-Fd, was potent against both gram-negative E.coli (MIC 2.5 µM,MBC 5 µM) and gram-positive Methicillin resistant S.aureus (MIC 5 µM,MBC 7.5 µM). i-Fd retained some activity against E.coli (MIC 5 µM, MBC 25 µM) but was inactive against S.aureus. (2) Potency comes from targeting both membrane interior of cell. (2) In comparison to the subdual and sequential “membrane followed by cell interior” mode of action of the i-Fm, the strong and simultaneous “membrane along with cell interior” targeting by i-Fd potentiates and broadens its action across the gram-negative-gram positive divide.

All abstracts are listed in alphabetical order of the presenting author.

Designing Peptidic Magic Bullets Against Bacteria

Vaccination in Patients with Cancer: Strategies to Prevent Influenza and Pneumococcal Disease

SAFDR A

BACKGROUND: Cord-blood derived (CB) is an important source of mostly immature dendritic cells (DC) immunotherapy against influenza virus.

METHODS: Recombinant hemagglutinin (rHA) of H1N1 was expressed by vector isolated of S. maltophilia and the susceptibility patterns.

RESULTS: HA-specific lymphocytes demonstrate identifiable HLA-restriction, HA-primed lymphocytes (HLA DRbeta1*1503) from a different CB demonstrated a nine-fold increase in statistically large spots when restimulated with the DR15-262 peptide. These data suggest that 1 in 1,900 of the HA-specific T-cells were DR15-262 restricted in a highly-specific fashion. Total ELISPot numbers of peptide DR15-262 and the control peptide were statistically distinct as well: 100 vs 18. HA-primed/DR15-262 co-stimulated with HA-primed/DR15-262 or in conjunction with adenoviral hexon-primed (irrelevant) lymphocytes did not demonstrate significant numbers of IFN-γ-spots. DR CTs were also assayed for HA-specific cytotoxic T-cell effectors. Naive cord blood T-cells were stimulated three times with rHA-loaded dendritic cells; specific type of HA-loaded autologous DC was double that of uninfected controls at an E:T ratio of 5:1 (p<0.05).

CONCLUSIONS: The results demonstrate that, despite the generally naive CB lymphocytes, influenza HA-specific responses can be generated ex vivo and could be potentially be used to enhance immune reconstitution following allogeneic stem cell transplantation.
A Promising Future for Cancer Immunotherapy in an Oil-Rich Country like Kuwait: An Invitation for Pharmaceutical and Non-Pharmaceutical International Collaboration

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Kuwait ranks sixth in the world in terms of oil reserve and ranks third in terms of oil exporter worldwide. The population of Kuwait reached only one million in 2006. Accordingly, Kuwait is a very wealthy country, and the average income of the Kuwaitis is one of the highest in the world. Despite the above wealth, the incidences of cancer in the country increased by 300% in the past 10 years. Surgery, and chemotherapeutic and radiation therapy are still the traditional methods used to treat the disease in Kuwait. Immunotherapy is still lacking in this country. Accordingly, and in an attempt to introduce such therapy, we have been working for the past four years on establishing the immune profiles of the neoplasm excepted from the patients, as well as the immune profiles of the patients, peripheral blood. Some interesting findings were seen during my work, and they sound appealing to start a nucleus of cancer immunotherapy in Kuwait.

Based on my findings, I have contacted the senior Kuwaiti officials enquiring about possible funding of the above nucleus. The feedback was extremely positive, and I was officially promised to be given the funding needed and more. Therefore, I would like to use this coming prestigious EHRlich II, 2nd World Conference on Magic Bullets as a venue to invite pharmaceutical and non-pharmaceutical international institutions to participate in establishing cancer immunotherapy in Kuwait. I believe this will add to the international efforts which have been aiming at making the dream of Paul Ehrlich come true.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts

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Nicotinic acetylcholine receptor containing an alpha subunit: target for a magic bullet?

**SALMINE N** 1, GRADY SR 1, MARKS MJ 2, COLLINS AC 2

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Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric assemblies made up of different combinations of alpha- (ligand binding) and beta- (structural) subunits. The alpha4beta2 nAChR is the most widely distributed subtype in the central nervous system. Recently it has been demonstrated that the presynaptic nAChRs in the brain dopaminergic neuron terminals consist of at least five different functional nAChR subtypes, two alpha4beta2-type nAChRs (alpha4beta2a and alpha4beta2b) and three alpha6-type nAChRs (alpha6alpha4beta2, alpha6alpha4beta2a and alpha6beta2beta2) (reviewed in Grady et al. 2007). The alpha6-type nAChRs have raised considerable interest because of their differing pharmacological properties compared with alpha4-type nAChRs. The distribution of alpha4 nAChRs is fairly restricted to the dopaminergic nerve terminals and the optical tract in rodent brain. One of the alpha4 nAChRs (alpha4alpha4beta2beta2) has the highest sensitivity to nicotine of any native nAChR that has been studied, to date (Salminen et al. 2004, 2007). This implies that this particular alpha6-type of nAChR is the first receptor to be activated following the initial puffs of tobacco. Functional and binding studies have yielded readily measured differences in sensitivity to nicotinic agonists and antagonists among these five nAChR subtypes. Several research groups have demonstrated that chronic nicotine treatment induces the downregulation of the alpha4 nAChRs while it is well established that other nAChRs (alpha4beta2a and alpha4b) are upregulated following chronic nicotine administration. Therefore, there is a reason to suggest that while alpha4 nAChRs differ in many ways from the other neural nAChRs it could be possible to develop subtype selective compounds that would allow therapeutic manipulation of nAChRs in number of conditions such as Parkinsonism or smoking cessation.

Calreticulin, a novel B-cell molecular target in gastrointestinal malignancies

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Background: Calreticulin (CRT), a calcium binding protein and cellular chaperon is suggested to play a key role in the recognition of dying tumor cells by the immune system. Calreticulin is recognized by antibodies (Ab) of some patients with autoimmune mediated hepatic and intestinal diseases. In an attempt to analyze the immune reaction to CRT during carcinogenesis, we 1) quantified serum levels of anti-CRT Ab in newly diagnosed patients with primary hepato cellular carcinoma (PHC, n=41), pancreatic tumors (PT, n=55), colorectal carcinoma (CRC, n=30) and gall bladder tumor (GBT, n=27), and patients with high risk for PHC – patients with viral hepatitis C (VHC, n=18), and for PT – patients with chronic pancreatitis (PT, n=16), 2) analyzed B-cell epitopes of CRT.

Methods: ELISA assays with human recombinant CRT or its peptides as an antigen were used for quantification of serum anti-CRT Ab. Pepcscan method employing synthetic decapeptides of CRT was used for determination of immunodominant epitopes of CRT.

Results: Statistically significantly elevated levels of anti-CRT Ab were found in patients with PHC and PT (IgA, IgG, P<0.001), ICA (IgA, P<0.001) and IgG (IgG, P=0.001) when compared with healthy controls. Interestingly, statistically significantly higher levels of IgA anti-CRT Ab were found in sera of patients suffering form PHC in comparison with VHC patients. Significantly higher levels of IgA Ab against CRT peptide KGEWKPRGSD (frequently recognized by IgAb of oncological patients tested in Pepcscan experiments) quantified by ELISA confirmed immunodominance of this peptide for PHC and PT.

Conclusions: 1) We revealed a developed immune reaction against CRT including affinity maturation and isotype switching in patients with gastrointestinal malignancies. 2) IgA and IgG anti-CRT Ab were predominantly detected in newly diagnosed patients with PHC, while 3) patients with PT – representing a risk group for development of PHC – produced only low levels of anti-CRT Ab.

New Approaches in the Treatment of Lymphoma Patients, R-Chop: Rapid Infusion Rituximab

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Background: Rituximab is a well tolerated treatment, but a cautious administration schedule is recommended to avoid infusion reactions. Rapid infusion over 90 minutes is feasible and safe. Since Rituximab is usually linked to other drugs with known myocardial toxicity, such as adriamycin, we decided to check the safety of a rapid infusion regimen and the presence of clinical or sub-clinical cardiac toxicity related to this method of administration.

Methods: We treated 42 patients with non-Hodgkin lymphoma with rapid infusion (over 60 minutes) Rituximab-based chemotherapy (CHOP-R), with average of 6.5 infusions of Rituximab per patient. Infusion-related events were analysed. We measured basal left ventricular ejection function (LVEF), every six months after chemotherapy, and the incidence of adverse cardiac events to assess cardiac toxicity.

Results: We had no grade 3 or 4 infusion-related events and no increased incidence or minor reactions during rapid infusions. None of the patients experienced cardiac events nor symptoms of cardiac failure during the whole observational period. Decreased in the post-treatment LVEF of over 10% was observed in 13 patients, and those with drop > 10% recovered normally. Patients with a LVEF decreased by 15% did not recover their normal level.

<table>
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<th>LVEF AFTER TREATMENT</th>
<th>Number of patients</th>
<th>(%)</th>
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<td>20</td>
<td>48%</td>
</tr>
<tr>
<td>Decreased (&lt;10%)</td>
<td>4</td>
<td>10%</td>
</tr>
<tr>
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<td>26%</td>
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<tr>
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Conclusions: Rapid infusion Rituximab is safe and well tolerated and when add to chemotherapy does not cause clinical cardiac toxicity although some subclinical decreased in LVEF can be observed.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
Telomerase as a Possible new Target for Cancer Treatment

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Background: Telomerase is a unique reverse transcriptase. Its main role is the protection and maintenance of telomeres, nucleoprotein structures at the end of linear chromosomes.

Most human somatic cells that do not or express low amounts of the enzyme and enter replicative senescence once their telomeres are short and signal a DNA damage response. In contrast, tumour cells and around 85% of cancer express high levels of telomerase activity constitutively. This contributes to unlimited proliferation and cellular immortality. The broad expression of the enzyme in many cancer entities makes it an attractive target for the development of new anticancer drugs.

Methods: We have designed hammerhead ribozymes against hTERT, the catalytic subunit of telomerase. We treated various breast and ovarian carcinoma cell lines with the ribozyme. Using isogenic systems of telomerase negative and hTERT-overexpressing fibroblast we demonstrate that telomerase protects cells from apoptosis. Also, clinically relevant drugs such as topoisomerase I inhibitors, if telomerase is inhibited or withdrawn from tumour cells these cells have an increased sensitivity to apoptosis and DNA damaging drugs.

Results: We found that cells treated with anti-hTERT ribozymes go into fast apoptosis. Many other approaches using different agents such as anti-sense molecules, chemotherapeutical inhibitors or dominant-negative mutants of hTERT rely on the effect of telomere shortening, which means that tumours with long telomeres have to go through many rounds of cell divisions until they finally end up with short telomeres and eventually go into apoptosis or senescence. We believe that in our case, the withdrawal of telomerase functions in a telomere-independent manner. We and other groups could show that telomerase inhibition has additional functional in addition to telomere maintenance. We could show recently that telomerase enters mitochondria upon stress and suggest that this could contribute to an increased resistance of cancer cells to apoptosis-inducing and DNA damaging chemo- or radio-therapeutic drugs.

Conclusion: Telomerase proves to be an important and exciting new target for the development of chemotherapeutic agents and anti-neoplastic drugs. Telomerase-inhibitors, developed by other groups and companies are undergoing clinical trials at the moment and are recommended as new, highly specific anti-tumour agents.

Production of cysteine rich peptides and protein in E. coli: hepcidin, the iron-regulating hormone

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Background: Hepcidin is a liver produced cysteine-rich peptide hormone that acts as the central regulator of body iron metabolism. Hepcidin is synthesized under the form of a propeptide, prohepcidin, which is processed to produce the biologically active mature 25 amino acid peptide. This peptide is secreted and acts by controlling the concentration of the membrane iron exporter ferroportin on intestinal enterocytes and macrophages. Hepcidin binds to ferroportin, inducing its internalization and degradation, thus regulating the export of iron from cells to plasma. The aim of this work was to develop a novel method to produce human and mouse recombinant hepcidins, and to compare their biological activity towards their natural receptor ferroportin.

Methods: Human and mouse hepcidins were produced in E. coli as Thioredoxin fusion proteins. Upon cleavage peptides, were purified by HPLC and characterized. The biological activity was measured on macrophages, following the ferroportin degradation induced by hepcidin.

Results: Human and mouse hepcidins, purified after cleavage from thioredoxin, were properly folded and contained the expected 4-disulfide bridges without the need of any renaturation or oxidation steps. Hepcidins were found to be biologically active, promoting ferroportin degradation in macrophages. Importantly, biologically inactive aggregated forms of hepcidin were observed depending on purification and storage conditions, but such forms were unrelated to disulfide bridge formation. Moreover this strategy was extended to the production and purification of prohepcidin, the 61 amino acids precursor of hepcidin. Prohepcidin was also found to contain the 4 disulfide bridges and able to generate biologically active hepcidin upon cleavage by furins (in vitro and in vivo).

Conclusions: Biologically active hepcidin and prohepcidin (its natural precursor) were produced in E.coli.

Abstracts
Albendazole Sulphoxide Levels in Endemic Normals and Filariasis Patients from a Lymphatic Filariasis Endemic Region of India Administered with Albendazole Using Liquid Chromatography

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Background: Determination of albendazole (ABZ) and its metabolites, albendazole sulphoxide (ABZSO) and albendazole sulphone (ABZSO2), in biological fluids is important to optimize dosage, length and frequency of therapy for the treatment of lymphatic filariasis. Aim: 1) To develop a simple and sensitive liquid chromatographic method for ABZSO determination. 2) To determine ABZSO in plasma of endemic normals. 3) To determine ABZSO in plasma of lymphatic filariasis patients following oral administration of 600 mg of ABZ.

Methods: A simple and sensitive reversed-phase isocratic high performance liquid chromatographic (HPLC) method for the determination of ABZ, ABZSO and ABZSO2 has been developed. The mobile phase consisting of acetonitrile-water-perchloric acid (70%) (30:110.0:0.06 (v/v/v)) was pumped at a flow rate of 0.80 ml/min on a 5 µm, reverse phase, Discovery® RP18-C18 column with UV detection at 290 nm. The calibration graphs were linear in the range of 0.05–1 µg/ml for ABZ, ABZSO and ABZSO2. The limit of quantification was 50 ng/ml for ABZ, 25 ng/ml for ABZSO and 30 ng/ml for ABZSO2. The within-day and day-to-day coefficient of variation averaged 4.98 and 6.95% for ABZ, 3.83 and 6.83% for ABZSO and 3.44 and 5.51% for ABZSO2, respectively. The mean extraction recoveries of ABZ, ABZSO and ABZSO2 were 79.25, 93.03 and 88.78%, respectively. The method was applied to determine the plasma levels of ABZ in 10 healthy endemic normals and 10 lymphatic filariasis patients administered with ABZ during pharmacokinetic studies.

Results: The method is suitable for the separation and determination of ABZ, ABZSO and ABZSO2 in a single chromatographic run. ABZSO attains peak plasma concentration of 362.50 µg/ml within 2-4 hours in endemic normals while peak plasma concentrations were 814.02 µg/ml in lymphatic filariasis patients within 2 hours following an oral administration of 600 mg of ABZ.

Conclusions: The method satisfies the criteria required for an assay required for human pharmacokinetic studies. The study shows good relation between dose, plasma concentration of ABZSO and time which has therapeutic significance for the treatment of lymphatic filariasis.

Keywords: Albendazole sulphoxide, lymphatic filariasis and liquid chromatography

Evaluation of Ecotoxicological impact of xenobiotic contaminants in terms of cytochrome P450 induction in marine fishes

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The use of molecular biomarker in marine pollution monitoring has gained enormous importance because of the increasing trend of contamination of the coastal environment by highly persistent organic pollutants. Most of these contaminants are being biotransformed through the food chain posing a serious threat to human health on environmental carcinogenesis. Among the persistent pollutants polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenz-dioxin (PCDDs), polychlorinated dibenz furans (PCDFs) are well known for their toxic potentials. The accumulation of these contaminants into the tissues of marine organisms are detoxified by the induction of cytochrome P450 enzymes. In order to assess the extent of ecotoxicological impact of xenobiotic contaminants on the marine environment the induction of cytochrome P450IA1 was studied in the edible marine fishes (Mugil cephalus, Sardinella longiceps and Rastrelliger kanagurta) from the Arabian sea along the Goa coast in terms of hepatic EROD activities. The Goan coastal environment is greatly contaminated with oils because of extensive shipping activities pertaining to fishing trawler, tourist boats, cargo ships, passenger ships, iron ore carrier, burgers etc. The oil found in the contaminated water, sediments and biota were composed of mostly high molecular weight polycyclic aromatic hydrocarbons (PAHs) with wide variation (0.476–5.882µg/l in water, 1.342–5.104µg/l in sediments and 8.54–37.89µg/l in biota) along the coast of Goa. The variation of EROD activities in fishes were in the range of 0.4335–3.377 pmol/mg/min along the Goa coast clearly indicating the extent of contamination of the coastal water by xenobiotic compounds such as PAHs, PCBs etc. Apart from the oil contamination, the coastal water also received huge amount of industrial wastes containing various types of xenobiotic compounds from the peripheral industries. The enhanced hepatic EROD activities in edible marine fishes clearly provides early warning signal of environmental carcinogenicity as evident from the production of reactive oxygen species via cytochrome P450 enzyme induction leads to the formation of DNA adducts resulting into DNA strand breaks.
Malignant brain tumors are relatively rare but lethal cancers. The median survival of glioblastoma multiforme (GBM), the most common primary brain tumor in adults, is 12-15 months despite current state-of-the-art multimodal therapies. Recent elucidation of the molecular pathogenesis of GBM has led to a rational development of molecularly targeted agents ("magic bullets") as a novel treatment venue against this devastating cancer. Monoclonal antibodies and low molecular weight kinase inhibitors are the most common classes of molecularly targeted therapeutics. Most clinical trials of these agents as monotherapies have failed to demonstrate survival benefit in unselected GBM patient populations. Several strategies have been developed to circumvent the poor response to first-generation targeted agents in GBM. Such strategies may include inhibition of multiple targets by either multi-targeted ("magic shotgun") inhibitors or novel treatment combinations. Multi-modality combination of targeted agents with radiotherapy or chemotherapy may improve efficacy. Indeed, the most promising salvage regimen for progressive GBM at present seems to be the combination of a VEGF neutralizing monoclonal antibody, bevacizumab and chemotherapy, inotocan. This regimen is associated with remarkable radiographic response rate and significant survival benefit in patients with recurrent GBM. Recently, we have identified tumor molecular profiles that may predict radiographic response and survival benefit in patients treated with this combination regimen. High tumor VEGF expression was associated with increased likelihood of radiographic response, while tumor hypoxia as measured by high expression of carbonic anhydrase IX predicts poor survival outcome. Future development of these "magic bullets" for GBM will require advances in discovery and validation of new molecular targets, improvement of therapeutic delivery and identification of biomarkers of response or resistance. Subsequently, each patient may be treated with personalized "magic bullets" based on molecular or genetic signatures. Possible role for human leukocyte antigen haplotypes in hepatotoxicity and/or pancreotoxicity associated with chemotherapy

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Background: Drug-induced liver injury (DILI) and drug-induced pancreatitis (DIP) are major health problems worldwide and leading causes of acute liver failure or severe pancreatitis. In addition, DILI and DIP are the main reasons for post-marketing regulatory decisions including withdrawal. Currently, there are reliable markers for the diagnosis of DILI or DIP. The identification of patients who are more susceptible to these unpredictable, idiosyncratic forms of adverse events is much needed. Genetic factors for susceptibility to DILI or DIP are receiving increasing attention.

Methods: Human leukocyte antigen (HLA) was analyzed by a standard microlymphocytotoxicity method in patients with DILI and/or DIP associated with chemotherapeutic agents. We elucidate the immunogenic predisposition of DILI and/or DIP.

Results: A case of terbinafine-associated fulminant hepatitis without pancreatitis showed HLA haplotype-A26, -A33, -B62, -B56, -DR9, and -DQ1. A case of micafungin-associated DIP without hepatitis showed HLA haplotype-A3, -B44, -B54, -B60, -DR9, and -DQ1. A case of trimethoprim-sulfamethoxazole-associated hepatitis and pancreatitis showed HLA haplotype-A2, -B24, -B56, -B62, -DR9, and -DQ1. A case of trimethoprim-sulfamethoxazole-associated hepatitis and pancreatitis showed HLA haplotype-A2, -B24, -B56, -B62, -DR9, and -DQ1. A previous case shows that the exact same HLA haplotype-A33, -B44 and -DR9 is detected in a case of nifekalant-associated pancreatitis and cholestatic hepatitis and case series of topotecan (tropotecanol[polyglycolone])-associated intrahepatic cholestasis. Moreover, case series of ticlopidine-induced hepatotoxicity are associated with the same specific HLA haplotype-A33. All taken together, HLA haplotype-A33 may also be important for DILI associated with chemotherapy. On the other hand, HLA haplotypes-DR may be common between our two cases of pancreatitis, suggesting that HLA haplotypes-DR and -DQ are linked to DIP.

Conclusions: HLA haplotypes-A33 and -DR9/DR14 may be associated with DILI and DIP, respectively. Because our presented cases are all Japanese, it is unknown whether the racial difference is involved in the pathogenesis of DILI and/or DIP besides on HLA haplotypes. Further studies regarding HLA haplotypes in patients with DILI and/or DIP regardless of race are much needed to avoid unpleasant hepatotoxicity and pancreotoxicity associated with drug therapy including chemotherapy.
Intraluminal Cefotaxime – Heparin Lock Placement in the Primary Prevention of Hemodialysis Catheter-Related bloodstream infections among the Elderly and Diabetics

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Background: Tunnelled-cuffed catheters (TCCs) are often used in the elderly and diabetic end stage renal disease (ESRD) patients, to carryout hemodialysis (HD). Complications like infection and thrombosis reduce the life-span of TCCs. Aim: To investigate the efficacy of cefotaxime - heparin lock in primary prevention of thrombotic and infectious complications and enhancement of TCC’s survival in the elderly and diabetic ESRD patients.

Methods: Prospective, randomized double-blind clinical trial I- TCCs (n=119), placed among 113 elderly patients requiring long-term HD were randomized to either group-I having TCCs (n=50, placed in 58 patients) ‘locked’ with cefotaxime (10mg/mL and heparin (5000 U/mL) or group-II with TCCs (n=50, placed in 55 patients) having catheter-restricted filling of heparin (5000 UI/mL) alone. Prospective, randomized double-blind clinical trial II- TCCs (n=109, placed among 96 diabetic patients requiring long-term HD were randomized to either group-I having TCCs (n=51, placed in 49 patients) ‘locked’ with cefotaxime (10mg/mL and heparin (5000 U/mL), or group-II with TCCs (n=58, placed in 47 patients) having catheter-restricted filling of heparin (5000 U/mL) alone.

Results: Trial I - Elderly patients with intraluminal cefotaxime / heparin lock on cumulative survival analysis test showed higher thrombosis-free TCC survival (84.7% vs. 63.3%, P=0.021), infection-free survival (68.7% vs. 31.3%, P <0.001) and infection-and thrombosis-free survival (65.5%, vs. 35.0 %, P=0.006) at 360 days in group I compared with group II. Trial II - Diabetic patients with intraluminal cefotaxime / heparin lock, on survival analysis showed a superior thrombosis-free (88.6% vs. 63.3 %, P=0.022), log rank) infection-free (72.9 % vs. 77.1 %, P=0.004, log rank) and thrombosis and infection-free TCC survival (78.4% vs. 37.9%, P=0.001, log rank) at 360 days besides having significantly lower incidence of CRBSI (3.68 vs. 1.56 episodes /1000 catheter-days, P <0.0001) and CRBSI-related mortality (23.4 vs. 9.8 %, P=0.015), compared with heparin-alone group.

Conclusions: Intraluminal cefotaxime-heparin lock safely and effectively enhance the life-span of TCCs by lowering the incidence of thrombotic and infectious complications, among the elderly and diabetic ESRD patients.

Breast and Prostate Tumor Cell Death by Genetically Altered Salmonella with Preferential Targeting of Mitochondria

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Background: Genetically modified Salmonella typhimurium strains present an attractive novel treatment for cancer, as Salmonella preferentially replicate within live cells.

3T3 cells were used to study the effects of Salmonella infestation on mitochondria in live cells. It also demonstrated the production of nitric oxide (NO) via induction of iNOS activity is involved the downregulation of the proapoptotic Bcl-2 family member BIM and involved the upregulation of its anti-apoptotic counterparts Mcl-1 and Bcl-xL.

Results: Our data show that genetically modified S. typhimurium (VNP20009 and CRC1674) destroy PC-3M and MCF-7 breast cancer cells with obvious destruction of mitochondria while the nucleus does not appear affected.

Depending on PKC-theta Expression, the Novel PKC Activator PEP005 can Either Increase or Decrease Apoptosis of Hemopoietic cells

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Background: Broad range activators of PKC isoforms induce apoptosis in many cancer cell lines. In T cells, however, activation of PKC can be a survival signal and a mitogen. We have been investigating the underlying mechanism explaining the difference between T cells and other hemopoietic cell lines in their response to PKC activators.

Methods: Activated CD8+ T cells rapidly enter apoptosis when deprived from growth factors such as IL-2 or IL-15. We cultured human resting and activated CD8+ T cells in the presence and absence of IL-2 and PEP005 and investigated the effects of PEP005 on cell-survival and proliferation. Furthermore we studied the effect of PEP005 on activation and expression of factors involved in regulation of apoptosis such as NFkB, PEP005 as well as Bcl-2 family members BIM, Bcl-xL, Bcl-2 and Mcl-1. We compared the response of CD8+ activated T cells to PEP005 to that of promyelocytic cell line HL-60, which does not express PKCθ.

Results: We found that PEP005, a novel PKC activator, can reverse cytokine as well as a survival signal. In freshly isolated naive or central memory T cells, PEP005 inhibits apoptosis and induces proliferation. We demonstrated that the survival effect depends on activation of PKCθ. Expression of this PKC isoform is largely restricted to T cells and myocytes. Our findings suggest that incubation of T cells with PEP005 inhibits apoptosis through activation of NFκB downstream of PKCθ. It also involved the downregulation of the proapoptotic Bcl-2 family members BIM and upregulation of its anti-apoptotic counterparts Mcl-1 and Bcl-xL.

Conclusions: We conclude that PKCθ expression determines if PKC activation increases or decreases the rate of apoptosis in haemopoietic cells.

Authors’ disclosure statement (not counting towards the character count).

This work was supported by the University of Birmingham, the Antibodies Research Campaign, the EU (FP5) and Peplin Inc.
Rimonabant, First CB1 Receptor Antagonist, a Potential Magic Bullet to Reduce Cardiometabolic Risk in Patients with Abdominal Obesity

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Targeting of nitric oxide donors to the phagosomal compartment of macrophages and the release of NO mediate killing of intracellular Leishmania parasites within the host cell

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Background: Abdominal obesity is associated with type 2 diabetes and dyslipidemia, leading to an increased risk of cardiovascular disease. The endocannabinoid system is overactivated in presence of abdominal obesity, which contributes to increased cardiometabolic risk. We describe the clinical results obtained with rimonabant, the first selective cannabinoid type 1 (CB1) receptor antagonist, in overweight and obese patients.

Methods: Rimonabant (6 and 20 mg) was first evaluated in the RIO programme: RIO-Europe, RIO-North America and RIO-Lipids in non-diabetic patients, RIO-Diabetes in patients with type 2 diabetes on monotherapy with sulfonylurea or metformin. Rimonabant 20 mg was further developed in type 2 diabetic patients treated with diet alone (SERENADE) or with insulin (ARPEGGIO), in abnormally obese patients (ADAGIO) and in patients with coronary atheroclesis (STRADIVARIUS).

Results: Rimonabant 20 mg, compared to placebo, consistently induced greater reductions in body weight, waist circumference, blood pressure, triglycerides, metabolic syndrome, insulin resistance and inflammation markers, and greater increases in HDL cholesterol and apolipoprotein concentration. In addition, in PI-diabetic population, rimonabant 20 mg was associated with a greater reduction in glycated haemoglobin (HbA1c) levels whatever the baseline therapy. Almost half of the metabolic effects of rimonabant were beyond weight loss. Favorable metabolic effects observed after 1 year persisted after 2 years. Rimonabant also reduced triglyceride levels, increased HDL cholesterol and adiponectin concentrations and, in addition, in PI-diabetic population, rimonabant 20 mg was associated with a non significant reduction in percent coronary atheroma volume, but a significant reduction in total atheroma volume (STRADIVARIUS). Adverse events more frequently reported with rimonabant were gastrointestinal, neurologic and psychiatric in nature, contraindicating the use of rimonabant in case of depression.

Conclusion: In both non-diabetic and diabetic overweight/obese patients, rimonabant 20 mg daily significantly improved the cardiometabolic profile via pleiotropic central and peripheral effects. Whether CB1 receptor antagonist might be considered as a "magic bullet" capable of reducing the incidence of cardiovascular events in this high risk population is currently evaluated in the large CRESCENDO outcome prospective trial.

A Role for Macroautophagy in Antiestrogen Resistance

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Background: The finding that, during therapy, tumors' sodium content correlates with corresponding alterations of water diffusion is attracting keen attention in the efforts to understand and further develop surrogate MRI biomarkers for tumor therapy. It is especially noteworthy that changes in both sodium and diffusion take place in the first days after therapy. Both types of imaging are able to detect a heterogeneity of tumor response and have potential to predict future individual tumor responses.

Methods: Rat 9L gliosarcoma cells were implanted intra-cranially or subcutaneously in male Fisher 344 rats (weight ~ 120 g). In ~14 days after tumor implantation, animals were subjected to a single ip BCNU chemotherapy with two different doses (13 & 26 mg/kg). Each 2-3 days, tumor growth, together with 3D sodium MRI and diffusion maps were detected following the treatments. The previous experiments were performed using 9.4T MRI scanner and the latest results were acquired using 21T magnet.

Results: The overview of sodium/diffusion MRI studies of rat glioma and 9L gliosarcoma tumors will be discussed, including the latest data acquired using the ultra high magnetic field of 21.1T available at NHMFL. Representative MR images of sodium and diffusion map acquired before and seven days after BCNU therapy (26 mg/kg) demonstrate dramatic increases in tumor sodium and ADC. At 21T, a unique sodium resolution of 1μm was attained (Fig. 1).

Conclusions: Sodium MRI and proton diffusion exhibit early and strong correlated responses in rodent models during tumor treatments equally in brain and subcutaneous tumors. Both methods revealed a decreased response if therapy doses were decreased or if tumor acquired an additional resistance. The ultra high field experiments at 21T allowed a record high resolution for sodium and demonstrated the potential of MRI to aid in tumor therapy. These two imaging modalities can be valuable biomarkers for individual evaluation of dramatic changes in vivo therapeutic cellular changes and for developing new drugs for tumor therapy.
High Throughput Screening for In Vitro Toxicity Screening: A Gradual Acceptance of New Test Methods

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Background: Early in vitro toxicity screening within the pharmaceutical industry has several advantages. A large number of compounds can be tested with small amounts of compound at a low cost price. Moreover deselective can take place at an early stage. The focus in this review is on genotoxicity, cytotoxicity, nuclear receptor activation as well as phase I and II enzyme competition assays.

Results: For genotoxicity, enzyme mutation and SOS repair are studied with Salmonella in Ames II (Kometix) or Vitox™ (Thermo) assays. Chromosomal aberrations are studied with a RAD54 promoter activation in yeast with Green Fluorescence Protein (GFP) Greenscreen (Gentrix) or β-galactosidase Radarscreen (RoMyrd) as read-outs. The Vitox™ assay is a predictive substitute for the full Ames test, while the Radarscreen assay is one for chromosomal aberration and micronuclei tests. For cytotoxicity, human liver HepG2 cells are used to determine glutathione depletion (Monochlorobismuth), calcine uptake (Caco2-AM), mitochondrial failure (Alamar Blue, O2 consumption LuxCell), DNA proliferation (Hoetch 33342), and radical oxygen species activation (Dichlorofluorescein) or NRF2 Responsive Element mediated luciferase activation.

For cytoxicity, human liver substitute for the full Ames test, while the RadarScreen assay is one for aberrations are studied with a RAD54 promoter activation in yeast with Green Fluorescence Protein (GFP) Greenscreen (Gentrix) or β-galactosidase Radarscreen (RoMyrd) as read-outs. The Vitox™ assay is a predictive substitute for the full Ames test, while the Radarscreen assay is one for chromosomal aberration and micronuclei tests. For cytotoxicity, human liver HepG2 cells are used to determine glutathione depletion (Monochlorobismuth), calcine uptake (Caco2-AM), mitochondrial failure (Alamar Blue, O2 consumption LuxCell), DNA proliferation (Hoetch 33342), and radical oxygen species activation (Dichlorofluorescein) or NRF2 Responsive Element mediated luciferase activation. These assays predict cytotoxicity towards a confidence level of 75%. Nuclear activation can be induced for the ArhydroxyCarbon Receptor (AhR), Pregnanе X receptor (FXR), Constitutive Androstane Receptor (CAR), Retinoic Acid Receptor α and β (RARs and R), Thyroid Receptor α (TRα), Liver X Receptor α (LXRα) or Farnesoid X receptor (FXR) in human liver HepG2 and/or H4IIE cells. This can be mapped among different strains of bacteria. However, there are still insufficiencies for the individual cytochrome P450 enzymes. Competition assays for Cytochrome P450 and UDP-glucuronoyltransferase are available and show good predictivity for human enzymes.

Conclusions: The strategy is to implement these assays in the early phase of toxicity testing. This means that all these the assays are performed for a ranking of the lead optimisation compounds. However, due to the high demands on purity of the compound for genotoxicity testing and the overall amount of compound needed for all these assays, i.e. 10 to 20 mg, in principle only after the first positive identification of the pharmaceutical activity in vivo and in vitro.

PKPD Modeling of Time-Kill Curves: Addressing Biphasic Killing Patterns

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Background: Bacterial time-kill curves can be normally modeled with simple modified indirect response Emax models. These models have been successfully applied to describe effects of β-lactams (penicillins, cephalosporins, and penem drugs) and a variety of strains of bacteria. However, some studies have been shown to exhibit a biphasic killing pattern, which cannot be described by the models applied in the past. In this work we evaluated different Pharmacokinetic/Pharmacodynamic (PK/PD) models to describe the biphasic killing pattern exhibited by ciprofloxacin (CIP), a fluorquinolone, in vitro time-kill experiments.

Methods: E. coli, P. aeruginosa, and S. aureus were exposed to changing concentrations of CIP in an in vitro model of infection, in which the 4 hour in vivo half-life of CIP was simulated. Static concentration killing curves for CIP were also constructed against E. coli and S. pneumoniae. In all experiments, samples were collected at predetermined time points. Three 10 to 100-fold serial dilutions were plated in duplicates and incubated overnight at 37°C. Time-kill curves were obtained by plotting the number of CFU/ml against time. Different PK/PD models were applied to describe the in vitro time-kill kinetics of CIP in these experiments.

Results: Biphasic killing pattern was observed against all three bacteria in the in vitro model of infection, as well as for E. coli in the static concentration time-kill experiment. The biphasic killing pattern against these strains was successfully described by both a novel Adaptive Emax model, which included a term to account for the change in the kill rate after approximately 4 hours, as well as a two-subpopulation Emax model. EC50 values of 0.0035 μg/ml for E. coli, 0.0129 μg/ml for P. aeruginosa, and 8.8 μg/ml for S. aureus were obtained. The model allowed good individual fits for multiple-dose data extracted from the literature. However, kill-curves against S. pneumoniae did not present the biphasic pattern, and were successfully modeled by the same modified indirect response Emax models applied to β-lactams.

Conclusions: 1) Biphasic killing kinetics was confirmed for CIP against 4 different strains of bacteria, but not against S. pneumoniae. 2) The biphasic killing pattern was described successfully by 2 very different models; 3) These results do not provide insight into the mechanism of this observation.

Bringing Light Into the Dark: Influence of Fluorescence Labeling on Protein Nanoparticles for in-vivo Use

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Background: Protein nanoparticles (NP) are one of the most promising tools for drug delivery in the field of tumor therapy. While scientific research advances from basic in-vitro tests towards complex in-vivo studies, the visualization of those NP within various animal systems becomes of increased interest. Several groups have used fluorescent dyes or quantum dots with the drawbacks of unintendedly changing the NP properties and very low signal intensities in-vivo. We investigated several labeling techniques and dyes for their influence on size, aggregation and surface charge.

Methods: NP were prepared from gelatin type A by two-step desolvation. Different commercially available fluorescent labels were incubated with the protein solution prior to NP formation or grafting to the NP. All covalent labeling steps were done according to the manufacturer’s protocol, while dextran dyes were incorporated into the gelatin matrix. Cationisation was done with cholate hydrochloride or with diethylaminoethanol-dextran. The read out of NP size and fluorescence intensity of the standards and NP was conducted with a fluorescence microscope. Size and zeta were analysed with dynamic light scattering prior and after each modification step.

Results: The signal intensity was comparable to the extinction coefficient from the dye manufacturers. The signal was factor 11.2 (SD 3.4) to 83.2 (SD 10.2) lower depending on the dye. NP in full blood showed even weaker fluorescent signals that could be increased by max. 24.5% with cell lysis. In a range of 0.02 mg/ml dye no significant changes in NP size and surface charge were observed if neutral dyes were used. pH sensitive dyes lead to an increase in NP size and a drop in zeta potential of the coated NP to the level of unmodified NP. Maleimide incorporation resulted in a reduction of the zeta potential but in a growth from 189 nm (SD 2.9) to 201 nm (SD 4.2). Statistical analysis of the data was performed by a one way analysis of variance.

Conclusions: 1) NP were labeled successfully with strong fluorescent dyes without major changes in the inherent properties at low concentrations and if applied prior to NP formation. 2) NP characteristics are influenced by the charge, steric properties and pH values of the used fluorophores. 3) Finally the signal intensity of our NP was maximized towards fast and reliable in-vivo detection.

All abstracts are listed in alphabetical order of the presenting author.
Bacterial Tetracycline Resistance: Prevalence, Evolution and Dissemination of Genes

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Background: Bacterial resistance to antibiotics is a global phenomenon. More than 40 distinct tetracycline resistance (Tc\(^\text{r}\)) genes confer resistance by four different mechanisms. Continually improving molecular and bacterial cultivation techniques have facilitated the identification of new genes in anarobic bacteria. Recently discovered mosaic Tc\(^{r}\) genes appear to have arisen by homologous recombination between parental genes, simultaneously present in the host bacterium. Mosaic genes are particularly prevalent in environments that have been gut ecosystems that are dominated by anaerobic bacteria. In contrast to tet(W), mosaic genes are less frequently found in oral samples compared to tet(W). PCR amplification utilizing full-length and nested primer sets for different Tc\(^{r}\) genes assessed the prevalence and distribution of mosaic genes.

Methods: A macroarray screen containing 23 prevalent tetracycline resistance (Tc\(^{r}\)) genes was hybridized to DNA extracted from human, oral, soil samples, and other sources of bacterial resistance. The gene cfr codes for a tRNA methyltransferase which targets the adenine residue at position 2305 in 23S rRNA. This 2.7kb region may be a mobile mini-element, and may be, very rarely among human and animal staphylococci, its location on plasmids points towards a potential for dissemination.

Results: In the presence of cfr, tet(W) can be reduced to ribosomes and modification in 23S rRNA in the presence of Cfr was investigated by OMTT-Microarray screens and primer extension analysis. The type of modification was identified by MALDI-TOF mass spectrometry. MIC testing of cfr carrying strains followed the CLSI recommendations. Location of cfr on plasmids was shown by transfer experiments.

Conclusions: Mosaic genes have been identified by different research groups from different individuals, reflecting the different microbial communities present. Close analysis of mosaic genes combining tet(O) and tet(W) motifs illustrated that recombination hotspots exist. The most complex mosaic gene identified was tet(OW32/O1W/D). Mosaic genes can be converted into parental genes by different recombination events. The most complex mosaic gene identified was tet(OV32/O1V/D).

Conclusion: The imatinib mesylate in continuous treatment in the CML allows often therapeutic breaks for major iatrogenic neutropenia. At the diagnosis, 33% of the patients had splenomegaly at diagnosis. The average number of leukocytes/mm\(^3\) was 187905 ± 75602.3 [55700-350000], the mean haemoglobin level was 9.4 g/dl ± 1.4 [5.7 and 11.5 g/dl]. Dose of imatinib administrated varied between 200 and 400 mg of modafinil were analyzed without knowledge of drug administration. All 16 placebo urine samples were correctly classified as negative at a modafinil concentration <0.4 µg/mL. No interfering substances (>0.4 µg/mL) were found in plasma samples from 28 randomly selected individuals or in urine samples from 16 individuals not taking modafinil. Likewise, aspirin and acetaminophen did not interfere with the HPLC method. Phenylthiocarbamoyl acid and modafinil acid, a major metabolite of modafinil, could not be extracted with ethyl acetate, but could be extracted from plasma and urine with ethyl acetate:acetic acid, 100:1, v/v.

Conclusions: Modafinil could be accurately and reproducibly analyzed in both urine and plasma. The HPLC method can be used for therapeutic drug monitoring, pharmacokinetic studies, and for drug abuse screening.
Effect of Pectin-Papain Interactions on Thermo-Mechanical Properties of Pectin Films Applied for the Treatment of Skin Wounds

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Background: Pectin is a biodegradable and water-soluble polymer. In this work, pectin obtained from peels of the maracuya (Passion fruit) was applied. It was used as support for papain (EC 3.4.22.2) immobilization to make the films suitable for treatment of skin wounds with this enzymatic debridement agent. However, the mechanical properties of the films must be improved to obtain the material with higher resistance to break. In the present study the effects of pectin-papain interactions on enzyme stability and thermo-mechanical properties of films with and without glycérin (G) and polyvinyl alcohol (PVA) were evaluated.

Methods: Films were prepared using solution of 1% pectin, 1 mg/ml of papain, G at 0.75% (w/v) or PVA at 0.25% (w/v). The films were studied by Differential Scanning Calorimetry (DSC) and Dynamic Mechanical Analysis (DMA), as well as in enzyme stability study performed spectrophotometrically using casein as substrate. Pectin-papain films with and without glycérin were tested on wounds of voluntary patients.

Results: Different glass transition temperatures (Tg) were detected: 18°C for pectin system, 15.43°C for pectin-papain, 18.48°C for pectin-G-papain and 25°C for pectin-PVA system. The values of the tensile breaking for pectin and pectin-papain films were 9.16 and 8.88 MPa, respectively, with an elongation at break of 1.643 and 3.85%, while for pectin-G-papain films were 14.2 MPa and 9.99%, 11.2 MPa and 2.68% in the presence of PVA. The PVA (but not G) decreased papain activity and stability. Moreover, in assays performed on the voluntary patients treated with pectin-PVA films, healing of the wounds were accelerated without any negative secondary effects.

Conclusions: 1. Addition of plasticizers improves the mechanical properties of the pectin films. The comparison of thermo-mechanical parameters demonstrates the presence of interactions leading to a change of mobility of biopolymer chains. 2. Glycerin addition does not influence on pectin-papain film capacity to accelerate healing of the skin wound.

Neurotoxicity Related to Lithium Combination Treatment in a Patient with Schizoaffective Disorder

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Background: The therapeutic efficacy of lithium in the treatment of affective disorders is invaluable. Nevertheless neurotoxicity should be considered as a significant side effect. Neurotoxic encephalopathy has been described in the literature both in lithium mono-therapy with normal serum levels or with toxic levels of lithium. Neurotoxic encephalopathy related to lithium – risperidone neuroleptics. In the following the case of a patient with schizoaffective disorder is presented. The relevant theoretical considerations of the aetiopathogenetic mechanisms are discussed, differential diagnostic steps and therapeutic implications are described.

Methods: A 60 year-old patient with schizoaffective disorder showed an acute neuropsychiatric state with severe cognitive deficits and an akinetic extrapyramidal syndrome under combined lithium risperidone treatment. An EEG investigation showed a marked change in basic activity. The clinical state slowly began to improve after withdrawal from the psychopharmaceutics, and the EEG also showed a clear improvement. The development of a neurotoxic encephalopathy under treatment with lithium and risperidone is the result of different aetiopathogenetic mechanisms. The most important hypothesis put forward is that lithium-neuroleptic treatment causes neurotoxicity by increasing dopamine receptor blockade which results in profound dopaminergic hypofunctionality reflected e.g. by extrapyramidal symptoms. The development of the syndrome can be said to be the most important differential diagnosis.

Conclusions: 1) Neurotoxic symptoms under lithium combination treatment may be interpreted as the interaction of different aetiopathogenetic mechanisms. Interactions in the dopaminergic system are very likely to play an important role. 2) The EEG is the most important diagnostic parameter for both the acute phase and for the follow up. 3) In patients developing signs of intoxication under lithium therapy, discontinuation of lithium medication should be considered.

Silkworm infection models to evaluate the therapeutic effects of antibiotics

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Most drug candidates obtained by in vitro screening are inappropriate as medicines due to their toxicity and their pharmacodynamics in the human body. Preclinical tests using animal models are essential, however, for evaluating the therapeutic effects of drug candidates for further development. Although mammalian models have been used to examine the pharmacodynamics of drug candidates, both the cost and the ethical issues of sacrificing mammals for drug analysis can delay the development of potentially therapeutic drugs. The use of invertebrate animals for the evaluation of drug candidates can overcome these problems. We propose the use of silkworms, Bombyx mori, as model animals to evaluate the properties of drug candidates. The lower cost and smaller space required for the maintenance of silkworms compared to mice allows for a larger number of animals to be handled in limited facilities. Because of the long history of the silk industry, the methods for taking care of silkworms are well established. Silkworms are ideal for use in a large-scale drug screening system, as they are large enough to be used in injection experiments, for making hemolymph preparations, and for isolating organs such as the midgut, which are essential processes for studying the pharmacodynamics of drugs in individual animals. The silkworm fat body functions in drug metabolism, similar to the mammalian liver, and contains a number of cytochrome P450s, sulfur or glucose conjugation enzymes, which are involved in drug detoxification. In this symposium, we report that pathogenic microorganisms, such as Staphylococcus aureus and Candida albicans, were lethal to silkworms, and clinically-used antibiotics had therapeutic effects in silkworms. Moreover, the effective doses of antibiotics in this silkworm infection model were similar to those in mammalian models. Further, the availability of antibiotics by oral administration, and the drug distribution and metabolism were similar between silkworms and mammals.
Inhibition of angiogenesis and melanoma metastasis by DisBa-01, an alphavbeta3-blocking RGD-disintegrin from Bothrops alternatus snake venom

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Background: The integrin v 3 is involved in multiple aspects of tumor angiogenesis and metastasis, which makes this receptor a key target for the development of anti-cancer therapies. Snake venoms are natural sources of small integrin ligands named disintegrins, which act as selective integrin inhibitors. We have previously demonstrated that DisBa-01, a recombinant RGD-disintegrin from Bothrops alternatus venom glands, is a strong inhibitor of the v 3 integrin, therefore inhibiting tumor cell adhesion to vitronectin (IC50 = 225 nM for B16F10 melanoma cells).

Methods: Inhibition of angiogenesis: The ability of DisBa-01 to inhibit bFGF-induced angiogenesis was tested in a matrigel plug assay in athymic nude mice. Inhibition of lung metastasis: The anti-metastatic activity of DisBa-01 was evaluated by injecting the luciferase-expressing B16F10-2BB cells mixed with DisBa-01 in the tail vein of C57Bl6J mice. The progression of pulmonary metastases was measured at day 1, 4, 7, 11 and 14 following cell inoculation by an imaging system. Flow assay: MDA-MB-231 cells labeled with cell tracker red were previously incubated with DisBa-01, mixed with whole blood labeled with calcine green and incubated in a flow chamber at 37°C for 15 minutes. The chamber contained collagen type I-coated coverslip. Adhered platelets and cells were differentially counterstained with the nuclear stain DAPI and visualized by confocal microscopy. Results: DisBa-01 dose-dependently decreased bFGF-induced angiogenesis in a matrigel plug assay (IC50 = 83 nM). When injected intravenously to C57Bl6J mice together with B16F10 melanoma cells, DisBa-01 time- and dose-dependently inhibited lung metastasis. Under flow conditions, DisBa-01 (100 nM) almost completely inhibited the adhesion of MDA-MB-231 cells to collagen I and to the extracellular matrix produced by endothelial cells as well. Detection of the N-terminal up to 26 residues did not affect the inhibitory activity of DisBa-01 to the v 3 integrin.

Conclusions: DisBa-01 is a potent new inhibitor of v 3 integrin-dependent adhesion processes involved in tumor angiogenesis and metastasis.

Support: FAPESP, CNPq (Brazil) and INSERM (France)

Correlation Between Clinico-pathological Features and Allergic Loss at TP53 In Metastatic Endometrial Cancer

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Adenocarcinoma of the endometrium (EC) is one of the most common malignancies of the female genital tract, with 4195 new cases diagnosed in 2005 in Poland. Although our knowledge of genetic features in primary endometrial carcinoma has been expanding, there is limited number of studies evaluated the role of molecular alterations during EC spread. Currently, we examined the allelic loss at the TP53 gene in 38 metastatic ECs, and investigated the relationship between LOH (Loss of Heterozygosity), p53 protein overexpression and clinicopathological features of cancer. Three intragenic TP53 polymorphic markers, located at intron 1, intron 4 and exon 4, were analyzed. Overexpression of p53 was evaluated immunohistochemically applying monoclonal mouse anti-human p53 antibody (clone DO-7, diluted 1:100) and the Vector Laboratories visualization systems. There was no significant association between LOH at intron 1 and clinical and pathological variables of cancer. A significant correlation existed between allelic loss at intron 4 and the presence of the neoplasm in the uterine cervix (R=0,319, p=0,049; Spearman rank correlation test). There was also a tendency for an inverse correlation between allelic loss at exon 4 and vascular space invasion, but this difference did not reach a significant value (R=0,321, p=0,068; Spearman rank correlation test); p53 protein was overexpressed in 34% (13 out of 38) ECs, either in “loss of one” or “in metastatic” endometrial lesions, and was significantly related to patients’ age (p=0,043) and to the presence of the neoplasm in the fallopian tube (p=0,046). Overexpression of the protein was significantly correlated with LOH at intron 1 of the gene (R=0,599, p=0,0001; Spearman rank correlation test), and a tendency existed for the correlation between p53 overexpression and allelic loss at intron 4 (p=0,059). Altogether, allelic loss at the TP53 is present in a subset of advanced-stage EC patients, and is correlated with p53 protein overexpression, particularly at intron 1 of the gene.

In Vivo Molecular Imaging of Capromab Pendetide in Humans and Small Animals Using Combined Dual-Modality SPECT/CT and microSPECT/CT Systems

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Background: Capromab pendetide is a monoclonal antibody targeting an intracellular epitope of prostate specific membrane antigen (PSMA). This antibody radiolabeled with 177Lu or 90Y for radioimmunoscintigraphy is primarily used for detection of lymph node metastasis or recurrence of prostate cancer. The same antibody also has a potential as a radioimmunotherapeutic agent with a radiolabel that emits beta particles such as 177Lu or 90Y. Single photon emission computed tomography (SPECT) has been the choice of imaging radiolabeled capromab pendetide. In comparison to standalone SPECT, a combined dual-modality SPECT/CT technology and radiolabeled capromab pendetide with anatomic details from computed tomography (CT) can offer a better image quality and quantitative accuracy of SPECT. Therefore, the aim of the study was to localize uptake of radiolabeled capromab pendetide with anatomic details from computed tomography (CT). Over the past decade, we have improved methods to image both humans and animal models using the SPECT/CT and radiolabeled capromab pendetide.

Methods: Patient capromab pendetide imaging studies first started in 1999 using a prototypic SPECT/CT system at UCSF. Since 2003 the studies were performed using a commercial SPECT/CT system that has a lower-resolution CT capability. Most recently in 2008, we started patient imaging studies with a high-end SPECT/CT system with a diagnostic-quality 16-slice CT capability. We started imaging LNCap xenograft mice using 111In- and 177Lu-labeled capromab pendetide using a dedicated small animal pinhole SPECT combined with CT system in 2005. We quantitatively analyzed interpreter’s confidence level for patient data when SPECT/CT technology was used, and pharmacokinetics and biodistribution studies were performed to localize uptake of radiolabeled capromab pendetide in the xenograft models. Results: We found an increased confidence level in interpretation of patient imaging data of 111In-capromab pendetide using SPECT/CT over SPECT. 177Lu-capromab pendetide showed similar or slightly better imaging characteristics over 111In-capromab pendetide in the LNCap models.

Conclusions: SPECT/CT imaging of capromab pendetide in humans improved the visual quality as well as our confidence in interpretation. The small animal imaging studies with both 111In and Lu as radionuclides showed a dual-potential use of 111In-capromab pendetide as a radioimmunotherapy agent and as an imaging agent during radioimmunotherapy.

Illicit Drugs and Cardiac Arrhythmias in Athletes

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The current management of athletes with arrhythmias is complicated by the large use of “illicit drugs”, taken at any age, by professional and non professional athletes.

The World Anti-Doping Agency (WADA) yearly updates a list of prohibited substances and methods banned by the International Olympic Committee. The list includes different classes of substances namely Anabolic Androgens Steroids, Hormones and Related Substances (Erythropoietin, Growth Factors, Mechanos Growth Factors); Gonadotrophins; Insulin; Corticotrophins, other Antidiabetics; Inducing substances and methods banned by the International Olympic Committee. The list includes different classes of substances namely Anabolic Androgens Steroids, Hormones and Related Substances (Erythropoietin, Growth Factors, Mechanos Growth Factors); Gonadotrophins; Insulin; Corticotrophins, other Antidiabetics; Inducing substances and methods banned by the International Olympic Committee. The list includes different classes of substances namely Anabolic Androgens Steroids, Hormones and Related Substances (Erythropoietin, Growth Factors, Mechanos Growth Factors); Gonadotrophins; Insulin; Corticotrophins, other Antidiabetics; Inducing substances and methods banned by the International Olympic Committee.

However, awareness of the current “2008” WADA list. The term “illicit drugs” comprises all categories of drugs banned by WADA, regardless of whether they are taken to improve physical performance (true “doping agents” or “performance enhancing drugs”) to mask the presence of specific doping agents during control tests (“masking agents”), or to counteract the hormonal side effects of doping agents. Several illicit drugs may cause cardiac collateral effects, through a direct or indirect cardiac action, and may provoke especially arrhythmogenic effects, during short, medium or long term.

The cardiovascular effects comprehend a wide spectrum of diseases: hypertrophic, dilated, ischimic cardiomyopathies, myocarditis, thrombo-embolic diseases and also a wide range of supraventricular and/or ventricular cardiac arrhythmias, focal or reentry type, that are often symptomatic and potentially lethal even in healthy subjects. The risk of lethal arrhythmias and sudden death (SD) is very high in subjects with preexisting cardiac diseases, particularly latent arrhythmogenic substrate or primary arrhythmogenic disorders including some inherited cardiomyopathies at risk of SD or with “no novo” structural disease due to assumption of the illicit drugs. Together with a continuous effort in improving the analysis of prohibited drugs it is crucial that the doping control strategies include the investigation of mechanisms of cardiac action and toxic effects of every single drug in the current “2008” WADA list.

Particular attention has to pay to “recreational drugs” (or drugs of abuse) including ecstasy and other amphetamines and numerous very new synthetically derived formulations, several classified ad “designer drugs”.

All abstracts are listed in alphabetical order of the presenting author.
Targeted Delivery of Cytotoxic Drugs by Means of Protein Vectors

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Background: Epidermal growth factor (EGF) and its receptor-binding fragment (EGFRf) were shown to be promising vectors for targeted delivery of cytotoxic agents to tumor cells. The use of short peptides as a vector component of the targeted preparations is more preferable owing to their high stability, easy availability and relatively low cost. The aim of the present study was design of vector peptides targeted to EGF receptor and investigating of cytotoxic activities of their conjugates with antitumor antibiotic doxorubicin (DOX).

Methods: Solid phase peptide synthesis (Fmoc strategy); study of growth-stimulating activities of peptides against fibroblast cell cultures; synthesis of DOX conjugates with peptides using crosslinking reagent; study of cytotoxic activity (CTA) of conjugates in vitro against human tumor cells.

Results: Two modified forms of EGFRf (EGFRf1 and EGFRf2) were synthesized. EGFRf1 differed from the original fragment by the presence of Ser instead of Lys in position 28. EGFRf2 contained Lys instead of Met in position 20 and Ser instead of Lys in position 28. Ser is one of the few amino acids forming the binding site in murine EGF. The undesirable conjugation at the α-amino groups of Lys in the active center of the receptor-binding fragment can be avoided through substitution of Ser for Lys, which, in turn, prevents the inhibition of binding of the polyepitope to the EGF receptor. The substitution of Lys for Met in EGFRf2 was performed to improve the conjugation of the peptide to DOX. Both EGFRf fragments manifested biological activities in vitro which exceeded activity of native EGFr. The conjugates manifested CTAs towards cultured human carcinoma cells HeLa which exceeded that of the free antibiotic 2.3-fold. The CTAs of the conjugates were close to that of free DOX against sensitive to this drug tumor cells MCF-7/M0 and exceeded 1.5-fold the CTA of DOX against resistant cells MCF-7-T2000 that characterized by hyperexpression of P170 protein.

Conclusions: 1) The amino acid substitutions in the EGFRf fragment are responsible for the increase of its receptor-binding ability. 2) Both peptides can be used for targeted delivery of DOX and, perhaps, some other antitumor drugs, to tumor cells.

Spectroscopic study on the interaction of the antitumoral drug emodin with bovine serum albumin: fluorescence, circular dichroism, SERS, SEF and stopped-flow techniques

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Background: Emodin shown an anti cancer effect in neuroectodermal tumor, breast cancer cells or prostate cancer. It binds Bovine Serum Albumin (BSA) forming a complex that is very important to ensure the drug delivery. Using Surface Enhanced Raman Spectroscopy (SERS), Surface Enhanced Fluorescence (SEF), Fluorescence, Circular Dichroism (CD) and Stopped-flow we have unequivocally characterized the binding sites of emodin/BSA complex.

Methods: SERS and SEF (U-1000 Jobin-Yvon Spectrophotometer provided by an argon ion laser (514.5 nm line)). Fluorescence, Perkin Elmer 50B with an excitation wavelength of 295 nm. CD: JASCO-710 spectropolarimeter between 200 and 240 nm. Stopped-flow: Biological SFM-3 system coupled to a transmittance detector.

Results: From SERS and SEF we deduced that in complexes, the neutral and mono anionic drug species are predominant. From fluorescence and CD we calculate the binding constants. CD results indicate a change in the α-helical contents of the protein when binding occurs. Stopped-flow experiments indicate the presence of two different mechanism of reaction for the binding.

Conclusions: 1) The primary interaction site of emodin is Sudlow’s site 2, where the bound drug presents a structure between neutral and mono anionic species due to the formation of some hydrogen bond. This interaction changes the α-helical contents of the protein. This process occurs for [emodin]/[BSA]≥2.0 ratios, and it implies a fast reaction with a complex mechanism of reaction, where the observed rate constant, kobs increases when [emodin]/[BSA] increases. After this interval, site 2 saturates. 2) The secondary interaction site of emodin to BSA occurs when [emodin]/[BSA]≥2.0 and corresponds to Sudlow’s site 1 binding. Drug species binding to this site is not exactly the same than that binding to site 2, and it exhibits a form more displaced to the neutral one. This interaction does not change the α-helical contents of BSA. This process implies a slow reaction with a different mechanism, where the observed rate constant, kobs is invariable when [emodin]/[BSA] increases.
The impact of a mixture of doxycycline, an acid and a detergent on root canal débridement

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Background: Pulp and periapical pathoses are microbial in nature. Removal of this flora is essential for healing. While débridement of the root canal system is achieved by mechanical instrumentation, supplemented with chemical disinfection, removal of the smear layer (created by the mechanical instrumentation) and the adherent bacteria are not consistently achieved. Here we have combined doxycycline with citric acid and a detergent (MTAD) that reduces surface tension thereby allowing enhanced penetration of the solution.

Methods: Part I (smear layer removal): Forty-eight extracted human teeth were prepared. Sterile water or 5.25% sodium hypochlorite (NaOCl) was used as an intracanal irrigant. Canals were treated with 5% of one of the following final rinses: sterile water, 5.25% NaOCl, 17% ethylenediaminetetraacetic acid disodium (EDTA), or MTAD. Presence or absence of smear layer and erosion of the root canal walls were examined under a scanning electron microscope. Part II (disinfection): Eighty-five extracted human teeth were contaminated with Enterococcus faecalis. After biomechanical instrumentation using 1.3% or 5.25% NaOCl, the teeth were exposed to a 5-min application of MTAD, 1.3% NaOCl, 5.25% NaOCl or a 1-min application of EDTA followed by 5% of 1.3% or 5.25% NaOCl. Teeth or dentin shavings were cultured for presence or absence of test bacteria.

Results: Part I (smear layer removal): MTAD was more effective in removing the smear layer and did not significantly change the structure of the dentinal tubules. Part II (disinfection): Fishel's exact test showed that combination of 1.3% NaOCl and MTAD was significantly more effective in disinfecting the root canal system than the other regimens. Chi2 test showed no difference among the other regimens.

Conclusions: Modification of doxycycline with citric acid and a detergent allows effective disinfection of the root canal system after short exposure. This contact disinfection might be partly due to acidity of the solution. Furthermore, the smear layer is removed without significant alteration to the dentin structure thereby enhancing the débridement process.

Authors' disclosure statement: The authors acknowledge that, as an institution, Loma Linda University retains a financial interest in this product.

Bacterial Drug Efflux Pumps: Significance for Antibiotic Resistance and Pathogenicity

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Background: The development and use of chemicals with antibacterial properties revolutionized the treatment of infectious diseases, but mechanisms of bacterial resistance to many antibacterials have evolved and threaten public health. Interestingly, the acquisition of resistance genes often decreases bacterial fitness in vitro, but little definitive information is available regarding their impact on bacterial fitness during infection. We examined this problem with the human pathogen Neisseria gonorrhoeae and an experimental murine infection model and tested if regulation of a multi-drug efflux pump can impact bacterial fitness. Methods: This study employed a murine vaginal infection and strains of N. gonorrhoeae bearing defined mutations in the mtrR promoter and at position -120 upstream of the mtrCDE operon; the latter gives evidence of generating a new promoter element. In contrast to mutations to antimicrobials and fitness in vivo but not in vitro. These mutations mapped to the transcriptional repressor gene mtrR, a 13 bp inverted repeat sequence within the mtrR promoter and at position -120 upstream of the mtrCDE operon; the latter gives evidence of generating a new promoter element. In contrast to mutations that de-repressed mtrCDE gene transcription, a null mutation in the mtrR gene decreased gonococcal fitness in vivo, but this could be counteracted by second site mutations that de-repressed mtrCDE expression.

Conclusions: The MtrC-MtrM-MHE efflux pump produced by gonococci is similar to other efflux pumps possessed by Gram-negative bacteria. We propose that this pump is required for gonococcal proliferation at mucosal sites and levels of its expression can significantly impact both antibiotic resistance and in vivo fitness.

Drug Cell Interaction at Molecular Level

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Background: We are using Patch clamp technique to measure the ionic currents involved in neuromuscular disorders and are looking at the drug cell interaction at the molecular level taking muscular dystrophy as an example. Many forms of muscular dystrophy are associated with a structural fragility of the muscle membrane, whereby membrane damage exceeds the ability of muscle to repair itself, resulting in the progressive degeneration of muscle fibers. Clinically and genetically, they are a heterogeneous group of inherited diseases. Mutations in several individual genes are now known to underlie the pathogenesis of different types of muscular dystrophy. Some of these gene mutations involve proteins expressed throughout the body, yet appear to preferentially affect skeletal muscle. We are interested to detect association of acetylcholine receptor protein with voltage gated sodium currents in biopsied cultured cells.

Methods: Whole cell and single channel configurations of patch clamp technique were used to measure the ionic currents gated by nicotinic acetylcholine receptors using acetylcholine and non depolarizing neuromuscular agent tubocurarine along with sodium channel agonist verapamil.

Results: Part I: (smear layer removal): MTAD was more effective in removing the smear layer and did not significantly change the structure of the dentinal tubules. Part II (disinfection): Fishel's exact test showed that combination of 1.3% NaOCl and MTAD was significantly more effective in disinfecting the root canal system than the other regimens. Chi2 test showed no difference among the other regimens.

Conclusions: Modification of doxycycline with citric acid and a detergent allows effective disinfection of the root canal system after short exposure. This contact disinfection might be partly due to acidity of the solution. Furthermore, the smear layer is removed without significant alteration to the dentin structure thereby enhancing the débridement process.

Authors' disclosure statement: The authors acknowledge that, as an institution, Loma Linda University retains a financial interest in this product.

Cytogenetic Response of Imatinib Mesylate in Chronic Phase Chronic Myeloid Leukemia

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Background: The chronic myeloid leukemia (CML) is characterized by presence of Ph chromosome (Ph+) in more than 90% of cases. The reciprocal translocation between chromosomes 9 & 22 results in the formation of Ph chromosome and a chimeric BCR-ABL fusion gene that causes CML. The disease follows a triphasic course – initial chronic phase (CP), accelerated phase (AP) and blast crisis (BC). Imatinib, a selective tyrosine kinase inhibitor, decreases the number of BCR-ABL positive colony formation in CML patients. Aims: 1) To perform chromosomal analysis and mutations were identified by DNA sequencing.

Results: imatinib reduced Ph chromosome in 23 (92%) out of 25 newly diagnosed patients. Cytogenetic studies were performed by GTG banding techniques. Karyotypes were interpreted according to the 1995 International System of Human Cytogenetic Nomenclature. Cytogenetic responses (CRs) were determined by the percentage of metaphase cells that were negative for the Ph chromosome.

Conclusions: Results: Out of 50 CML-CP patients 48 (96%) were Ph positive and 2 (4%) were Ph negative at the time of diagnosis. Out of 48 Ph positive patients who were under imatinib treatment, 25 patients were eligible for follow up. After a median follow up of 12 months, 16 (64%) patients had major cytogenetic response (Ph+ve cells less than 35%,Ph+ve cells more than 0% to 35%) in 15 (60%) and complete CR (Ph+ve cells 0%) in 4 (14%), 7 (28%) patients had minor cytogenetic response (Ph+ve cells 35% to 65%) and 2 (8%) patients showed imatinib resistance.

I matinib: Imatinib reduced Ph chromosome in 23 (92%) out of 25 newly diagnosed Ph positive CML patients in chronic phase.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
Intragraft Administration Of Abciximab And Verapamil Combined With Direct Stenting Prevents Slow-Flow And No-Reflow Phenomenon In Saphenous Vein Graft Percutaneous Coronary Intervention

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Background. Slow flow/no-reflow phenomenon (SF-NR) complicates up to 15% cases of percutaneous coronary intervention (PCI) in saphenous vein grafts (SVG). We hypothesized that a strategy of prophylactic intragraft administration of abciximab and verapamil into the SVG, combined with immediate direct stenting of the graft lesion without pre-dilatation, would reduce the risk of platelet activation, microvascular vasospasm and distal plaque embolization respectively and cause a reduction in the incidence of SF-NR.

Methods. Data from 130 consecutive patients who underwent PCI of SVG lesions in a single center over a 7-year period were reviewed. Patients who underwent conventional PCI technique (balloon pre-dilatation of the target lesion prior to stent deployment; optional use of intragraft verapamil or intravenous abciximab) were assigned to the control group (n=72). The patients who received prophylactic intragraft administration of abciximab (0.25 mg/kg) and verapamil (100-300 mcg, depending upon blood pressure and heart rate) through the guiding catheter followed by direct stenting were assigned to the novel strategy group (n=58). The primary outcome was the occurrence of SF-NR. Clinical endpoints included death, target vessel revascularization (TVR), and MACE during the hospitalization period, 30 days and at 1 year.

Results. SF-NR occurred more frequently in the control group compared to the novel strategy group (11% vs. 2%, P=0.04). One patient in the control group died after developing persistent SF-NR and acute MI post-PCI. No death was reported in the novel strategy group. Three patients, all in the control group, developed post-PCI MI during the index hospitalization. The difference in 30-day MI and TVR rates did not reach statistical significance. There was a non-statistically significant trend towards higher 1-year MI rate in the control group (8% vs. 2%; P=0.13). The control had significantly higher rates of MACE (25% vs. 7%, P=0.01) and TVR (22% vs. 7%, P=0.03) at 1 year as compared to the novel strategy group.

Conclusions: 1) Substantive improves human adaptation to nature. 2) Substance use and abuse act as a substitute for more serious deviance and even crime in a society. 3) Substance users serve their group, culture, and historical era as messengers of critical issues or innovators of alternative lifestyles.
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Background: As extended hepatectomy (HE) and chemotherapy including targeted agent has developed during the last several years, the treatment outcome for multiple liver metastases from colorectal cancer has improved. Aims: To clarify the current status of HE with chemotherapy.

Methods & Results: Extended HE 87 patients who underwent HE for multiple (>4) and bilobar liver metastases from colorectal cancer from 1992 to 2006 were enrolled in this study. Results: The mean resected volume of straightforward HE in 51 patients, HE after portal embolization (PE) in 13 and two-stage HE with or without PE in 23 was 401.9, 654.5 and 879.9 individually. There was no mortality nor significant difference of morbidity among them. The hypotrophy ratio of the future remaining liver volume (before/after the first procedure) in HE after PE, two-stage HE and two-stage HE with PE was 1.25, 1.25 and 1.6 individually. The 5-year overall survival rate and 5-year disease-free survival rate after HE were 38.0% and 11.7% individually. From these results, when the remaining cancer-free liver volume is only 200g, two-stage HE with PE should be employed. Perioperative chemotherapy

Conclusions: The treatment consists of systemic chemotherapy with 5FU+FA+CDPP or currently FOLFOX and hepatic artery infusion (HAI) with 5FU+FA+CDPP. 5-year survival rate of adjuvant systemic chemotherapy (49.9%) or adjuvant HA chemotherapy (64.3%) are significantly higher than that of ALST and ALT. The absence of less than 5g of total injected 5FU through the HAI. There was a significant difference in survival of patients with 6 or more tumors and CEA >11ng/ml between patients with and without neoadjuvant chemotherapy. In the 5-year overall survival rate of the responders consisting CR, PR and ST to the chemotherapy (45.8%) was significantly higher than that of the non-responders (22.6%). Downstaging chemotherapy provided 5-year survival rate of 34.7% after HE in 19 out of 138 patients (13.8%) with initially irresectable liver metastases.

The remaining cancer-free liver volume is less than 200g, two-stage HE with PE should be employed. Perioperative chemotherapy and extended HE enabled patients with multiple bilobar liver metastasis to survive a long period, specifically for patients of which tumor showed responsiveness to chemotherapy.

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Background: Recently, many ophthalmologists who specialize in retinal diseases have interests in macular edema (ME) which directly leads visual disturbance. Macular edema was often seen in vasoproliferative and ischemic retinal diseases as diabetic retinopathy (DR) and retinal vein occlusion (RVO), and when pathogenesis are related with VEGF expression, therefore anti-VEGF therapy is expected to be effective in ME. In this presentation, the effectiveness of intravitreal injection of bevacizumab, an anti-VEGF antibody against DR- and RVO-associated macular edema was evaluated.

Methods: 86 eyes of DR-associated ME and 75 eyes of RVO-associated ME participated in this study. After the initialocular examination, 1.25mg of 0.5 ml bevacizumab was injected intravitreally in each eye. ME was evaluated by measuring macular thickness with optical coherence tomography (OCT) for up to 24 weeks. Clinical course of best corrected visual acuity (VA) in each eye was monitored.

Results: Before starting this study, averaged macular thickness was 634.6 ± 96.5 m in DR-ME, and 662.1 ± 104.8 m in RVO-ME, which is no statistical difference between two groups. After the injection, averaged macular thickness in both diseases showed prominent decrease to 63.5 ± 14.2 2% in DR-ME and 40.7 ± 11.6 % in RVO-ME within 1 month. However 70 eyes of 86 (81.3%) DR-ME and 49 of 75 (65.3%) eyes of RVO-ME showed reduction of ME by 24 weeks. There is no significant difference of initial VA between both groups. After the injection, VA in both groups showed rapid and temporary improvement and after that it gradually decreased but kept significant improvement at 24 weeks. Temporal regression of VA in RVO-ME eyes was significantly better than that of DR-ME eyes.

Conclusions: Intravitreal injection of bevacizumab is possible to reduce macular edema, however, recurrence of the edema was observed in most cases. Although the other therapies including retinal photoocoagulation or vitreous surgery must be added, the rapid and prominent reducing effects by intravitreal bevacizumab prevent visual disturbance which is reflected in functional loss of the edema. The effect of bevacizumab against macular edema was more expected in RVO than in DR.

A Bevacizumab, An Anti-VEGF Antibody, As A MAGIC BULLET for Retinal Disease?

SHIMURA M

A MAGIC BULLET for Retinal Disease?

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A MAGIC BULLET for Retinal Disease?
Background: Both neoadjuvant and adjuvant chemotherapy, being carried out so far with cytotoxic agents, single or combined, are highly toxic to the patient because they distribute in the whole body. The lifelong experience of Paul Ehrlich for finding chemotherapy specifica agents had been based mainly on: i) healthy nutrition; ii) avoiding disease risk factors in life-style; iii) study of the cause of cancer; iv) finding a suitable non toxic pharmaceutical or radiopharmaceutical which can eliminate the cause of cancer. Epidemiological and etiological results for the incidence of different cancers are of great help in finding the cause of cancer.

Methods: We determine the total-body distribution of radiopharmaceuticals of known chemical composition, and study trace elements in the disease and also in the neighbouring healthy tissue with sector-field inductively coupled plasma mass spectrometry (SF-ICP-MS).

Results: Our study have shown that radiopharmaceuticals for cancer diagnosis and therapy are continuously changing their chemical composition after synthesis. They are therefore of little use for diagnosis and therapy. We are, at present, trying to develop chemotherapia specifica for safe and effective cure of bladder cancer patients. The risk factor for bladder cancer in the order of their decreasing harm are: i) cigarette smoking, ii) red meat consumption, iii) alcohol drinking, iv) coffee drinking.

Conclusions: The bladder cancer patients have to avoid these injurious substances as soon as possible when early signs of bladder cancer are observed. We have found how these risk factors work together to cause bladder cancer

Results of a series of pharmacoepidemiological studies. In the EuroSCAR study investigated a large number of patients with severe cutaneous adverse drug reactions in a series of pharmacoepidemiological studies. In the EuroSCAR study relative risks for different drugs to cause SJS/TEN and AGEP could be calculated for AGEP strongly associated drugs, i.e. drugs with a lower bound of the 95% confidence interval (CI) of the odds ratio (OR) > 5 were pristinamycin, ampicillin, quinolones, (hydroxy)chloroquine, anti-infective sulphonamides, and therapy. We are, at present, trying to develop chemotherapia specifica for safe and effective cure of bladder cancer patients. The risk factor for bladder cancer in the order of their decreasing harm are: i) cigarette smoking, ii) red meat consumption, iii) alcohol drinking, iv) coffee drinking.

Conclusions: The bladder cancer patients have to avoid these injurious substances as soon as possible when early signs of bladder cancer are observed. We have found how these risk factors work together to cause bladder cancer
Application of the Fluorescence Quenching Method to the Study of the Interaction of the Anti-psychotic Drug Chlorpromazine with Serum Albumin

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Background: The proper understanding of drugs binding to plasmatic proteins is an important pharmacological parameter determining their distribution, absorption and elimination, with possible undesirable clinical effects. The aim of this work is to study the binding of chlorpromazine (CPZ) with human and bovine serum albumin (HSA and BSA). CPZ is a neuroleptic still applied in psychotis treatments and its chronic use may cause severe side effects, such as parkinsonism and tardive dyskinesia. Here we present the Stern-Volmer constants, besides discussing the binding process.

Methods: Intrinsic fluorescence of BSA and HSA were measured by selectively exciting their tryptophan residues at 290nm. The gradual quenching was observed by the titration of both proteins with CPZ, and the Stern-Volmer graphs were plotted to evaluate the quenching constants.

Results: HSA titrated by CPZ yielded a linear Stern Volmer plot, whereas BSA yielded a slightly concave curve upward when CPZ concentration was higher than 10 M. Comparison of experiments with HSA carried out at 35º C and 25º C revealed an important deviation in the slope of the Stern-Volmer plot. For HSA, the Stern-Volmer constants were 4.1x10⁴ ±1.6x10³ M⁻¹ at 25ºC, and 6.7x10⁴ ±2.0x10³ M⁻¹ at 35ºC. The increasing of the angular coefficient caused by the higher temperature suggests the occurrence of dynamic quenching for HSA. The estimated constant for BSA was 3.3x10⁴ ±9.0x10³ M⁻¹ at 25ºC.

Conclusions: (1) CPZ quenches the fluorescence of HSA by collisional quenching, interacting with this protein without forming complex. (2) The primary binding site of CPZ is close to the single tryptophan residue of HSA, at the position 214, in the hydrophobic region of the chain. (3) CPZ binds to BSA near to the tryptophan residue located at the position 212.

Microdialysis with Moderately Lipophilic Drugs: What Is Essential to Know for Voriconazole and Its Drug Product to be Applicable in Pre-/Clinical Microdialysis Settings?

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Introduction: Microdialysis (µD) is regarded as method of choice for the determination of unbound tissue concentrations at target sites. Prior to its in vivo applicability in vitro investigations are crucial. For treatment of severe fungal infections voriconazole (VRC) presents a very potent antifungal. VRC combines several pharmacological properties for its therapeutic application: lipophility and low solubility. An i.v. formulation is available containing solubilizing sulphobutyl-β-cyclodextrine- sodium (SBECD). The aim was to investigate whether unfavourable properties of VRC allow the applicability and conditions of the µD technique in pre-/clinical pharmacoepidemiological studies.

Methods: A reliable and rapid HPLC assay for the quantification of VRC from small µD volumes was developed and validated. For the in vitro µD investigations a robust and easy-to-handle system was developed permitting physiological conditions. Experiments on the influence of flow rate (0.4-10.0 µL/min), VRC concentration (1.0-50.0 µg/mL), steady state conditions and VRC flow direction regarding the membrane on relative recovery (RR) were performed. In addition, the impact of SBECD in the i.v. VRC formulation was assayed under retrodialysis conditions.

Results: All validation parameters met the criteria set in the international FDA guideline for bioanalytical methods. In vitro µD experiments revealed no binding of VRC to any part of the µD device and therefore VRC concentrations achieved in the dialysate are considered to be sufficient for in vivo investigation. From the observed flow rate dependency a flow rate of 2 µL/min for optimal recoveries for future in vivo investigations was determined yielding adequate sample volumes >12 µL for HPLC assay. RR was independent of VRC concentration. Steady state investigations were performed for the first time and led to recommendations for the in vivo probe calibration solution concentration to range between 100 µg/mL and 200 µg/mL. An impact concerning recovery enhancement of SBECD in the drug product of VRC could be precluded.

Conclusion: The results present the essential prerequisites for further VRC investigations especially for the use of clinically approved Vfend® for determination of relative recovery in pre-clinical trials.
H3S-induced suspended animation during porcine aortic occlusion-induced ischemia reperfusion injury

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Background: In mice, inhalated hydrogen sulfide (H3S) produced a “suspended animation” with a characteristic pattern of hemodynamic alterations caused by hypotension and hypothermia, which protected against otherwise lethal hypoxia and hemorrhage.2,3 The large surface area / mass ratio, however, facilitates cooling in rotgers, and controversial data were reported on the effects of H3S in large animals.4 Therefore we investigated whether H3S may induce a similar metabolic response and thus be organ-protective during aortic occlusion-induced ischemia/reperfusion (IR) injury.

Methods: In a first series, 16 pigs undergoing 30 min of aortic occlusion received the i.v. H3S-donor NaH3 or vehicle started 2 h before ischemia and continued during the whole reperfusion period. During aortic occlusion mean arterial pressure (MAP) was maintained at 80-120% of baseline with esmolol, nitroglycerin and ATP, during reperfusion noradrenaline was titrated to keep MAP at baseline levels. In the second series, 19 pigs underwent prolonged (60 min) aortic occlusion to produce acute renal failure. In both studies the reperfusion period was 8 h.

Results: In the first study NaH3 significantly reduced heart rate and cardiac output without affecting stroke volume, significantly decreased the dose of noradrenaline required to maintain hemodynamic targets, and caused a significant drop in O2 uptake, CO2 production, and, consecutively, core temperature. These results were confirmed in the second study. Moreover, NaH3 was renoprotective as demonstrated by a significantly higher glomerular filtration rate and a lower serum creatinine level at the end of the reperfusion period, which coincided with a reduced oxidative DNA-damage in the kidney tissue as assessed by the tail-slip method in the alkaline comet-assay.

Conclusions: In vivo, NaH3 (i) reduces metabolism and energy expenditure in anesthetized large-animals, (ii) improves noradrenaline responsiveness during reperfusion after aortic occlusion, and (iii) protects the kidney against IR injury.


Acknowledgement: Supported by the DFG (SCHM 899/2-3)

Surface Modified Nanosuspensions of Nevirapine for Brain Targeting

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Background: AIDS is one of the most frightening syndromes worldwide with 5 million new HIV infections a year. Around 14000 people are becoming infected each day. About 10-15 million people around the world are infected with HIV and 5-6.8 million of AIDS by 2020. HIV potentially has the potential to destroy different reservoirs like CD4+ T lymphocytes, CNS, lymph nodes, GALT, genital organs, stomach, liver, eye & lungs. Antiviral drug delivery system of Nevirapine. Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against human immunodeficiency virus type 1 for brain targeting.

Methods: Nanosuspensions of Nevirapine were fabricated by high pressure homogenization technique applying 32 factorial design for intravenous application. Further Surface modification of nanosuspensions was done using PEG 300,400,1000, Poloxamer 188, Dextran 60 and bovine serum albumin by covalent conjugation. The developed nanodispersions were characterized for physiochemical parameters & stability. The nanoparticles were sterilized & converted into reconstitutable form. Formulations were tested for their anti-HIV potency, cytotoxicity and phagocytic uptake in J744.A1 cell line. The in-vivo tissue distribution was carried out in Wistar rats using gamma Scintigraphy.

Result and discussions: Nevirapine nanodispersions were successfully prepared. Homogenization parameters and concentration of surfactant had marked effect on particle size distribution. The nanoparticles with mean particle size of 482 nm and drug release with t50% of 0.22 h and 90% of 3.48 h were obtained. AFM confirmed surface modification with increase in particle size. Among cryptoprotecates, trehalose gave the desired particle size after reconstitution. Developed nanoparticles crossed BBB within 30 min and remained there up to 8h.

Conclusion: Thus we state that successfully developed Nevirapine surface modified nanocarriers for brain targeting.
Lipid Nanocarriers for delivery of antimalarial drug Primaquine

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Background: Primaquine(PQ), is the only available antimalarial that acts specifically on the pre-erythrocytic schizonts of plasmodium, which reside and multiply in live and is considered to be a crucial weapon against resurgent vivax malaria. PQ has limited bioavailability because of pre-systemic metabolism and excration and is characterized by severe toxicity when required in high doses to treat severe cases. Targeting of the drug to the site of action, liver, would possibly reduce the dose and overcome the toxicity problem

Methods: Lipid nanoemulsion and nanoparticles of PQ were prepared using highly specialized short and medium chain triglycerides with combinations of various hydrophilic and lipophilic surfactants & optimized by applying 3 factorial designs. Antimalarial activity was investigated using Peter’s four-day suppressive test using swiss albino mice. Parasitic culture of Plasmodium berghei yoelii was injected intraperitonially to induce malaria. After 4h of infection, the formulations were administered at 4 different dose levels, both by oral and parenteral route. From the 5-th onwards, blood smears were collected and observed for the presence of parasites. Parasitemia(%) and mean survival time(MST) was recorded.

Dose titrations and pharmacokinetic studies were performed in Wistar rats. Lipid nanocarriers were administered orally and intravenously and the animals were sacrificed at the stipulated time intervals and drug content in various organs was determined

Results: The nanocarrier systems showed a narrow particle size distribution with mean particle size ranging between 91-117nm, high drug entrapment and 100% drug release within 24hrs. In vivo, Control reduced RBG with 90% parasitemia by day 10 and no animal survival after day 11. The developed nanocarriers showed 3 times better suppression of parasitemia and higher MST at 25% lower dose as compared to conventional formulation. PQ showed improved oral bioavailability and higher levels in the liver after incorporation into lipid nanocarriers. Kidney showed much lower concentration of unmetabolized drug, indicating reduced nephrotoxicity with the developed formulations.

Conclusions: Thus lipid nanocarriers have high potential for the delivery of primaquine to liver with enhanced antimalarial efficacy and reduced toxicity.

The Application of Early Bactericidal Activity (EBA) studies in Assessing Antituberculosis Drugs

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Background: Alternative therapeutic regimens with new faster-acting drugs are needed to stop the spread of tuberculosis (TB). Quantitative and reproducible testing methods are vital to formulate appropriate treatments. Studies of the early bactericidal activity (EBA) were established as an initial step to assess new testing methods are vital to formulate appropriate treatments. Studies of the early bactericidal activity (EBA) were established as an initial step to assess new antituberculosis drugs. It measures the ability of individual antimicrobials to kill bacilli in cavities of patients with pulmonary TB at the beginning of therapy. Objectives: To retrospectively review the value of EBA studies to determine: 1) whether a drug has activity against bacilli in cavities of patients with pulmonary TB at the beginning of a 5-day study period are also include d. Dose titrations were done on H, R and RPE and therapeutic margin were estimated for H and R. EBA measures the decrease in log10-colony forming units (CFU) of M. tuberculosis per ml sputum per day. Sixteen hometrin sputum collections were homogenised, digested with sputatol, serial dilutions were plated onto selective TH11 agar plates, incubated at 37 °C for 3 weeks for CFU counts.

Results: The 0-2 day EBAs (and standard deviation) were: H 6 mg/kg, 0.50–0.64 (0.16–0.30) in 5 studies; R 12 mg/kg, 0.17–0.22 (0.05-0.25) in 3 studies; Z 40 mg/kg, 0.003–0.014 (0.025-0.14), E 25 mg/kg, 0.25–0.43 (0.16). HRZ in combination 0.508 (0.16). Succeeding 2-5 day EBAs were: H 0.06 (0.12); R 0.20–0.30 (0.11-0.18); Ofloxacin had a 0-2 day EBA of 0.39 (0.19) and a 3-5 day EBA of 0.17 (0.12). The 0-2 day EBAs for RPE at 600, 900 and 1200 mg were 0.244 (0.189), 0.364 (0.186) and 0.234 (0.199), respectively and the 2-5 day EBAs 0.193 (0.145) 0.251 (0.116) and 0.241 (0.112), respectively. The therapeutic margin for H was 300/15 = 20 and for RPE 150/5 = 30.

Conclusion: The EBA procedure requires small numbers of patients and can be carried out at relatively low costs to rapidly determine the therapeutic value, dosage and role of new drugs.

Targeting HIV Reservoir & Sanctuary Sites Using Peptide Backbone Polyethylene Glycol (PEG) Nanocarriers

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Background: Despite the wide variety of highly potent anti-HIV drugs that have been developed and brought to the clinic over the years, eradication of HIV infection has not been achieved. We have focused our efforts towards the design and development of PEG-nanocarriers for macrophage targeting. Macrophage targeting represents a key challenge in HIV therapy, since they are not only the primary target of HIV infection, but also provide reservoirs for the virus. CD4+ T-lymphocytes, they are an important source of HIV persistence.

Methods: A modular PEGylated peptide [acetyl-Cys-(Ala-(Ala-Cys)-(PEG2))] nanocarrier was designed and developed incorporating two and four copies of PEG(FK(frucosene))C. Two aspects were investigated: IMLF copy number required for optimal binding and optimal PEG size for macrophage uptake. One, two, and four-arm PEG scaffold of molecular weights 5, 10, and 40 kDa were used to conjugate up to four copies of IMLF(FK(frucosene))C. Nanocarriers were characterized using amino acid analysis, MALDI-TOF mass spectrometry, and Size-Exclusion chromatography. Receptor expression was confirmed using RT-PCR and Western Blot analysis. Macrophage-like differentiated human U937 cell-specific binding and cellular uptake studies were performed. Binding, avidity, and uptake were evaluated.

Results: Nanocarrier uptake was found to be energy dependant and mediated by the IMLF receptor. IMLF copy number was found to influence the binding and uptake behavior. Increasing the number of IMLF molecules from one to two resulted in enhanced uptake of 4-fold, but increasing PEG copy number from four led to an increase in the uptake but further increase to 40 kDa led to decreased uptake.

Conclusions: Receptor-mediated endocytotic uptake of nanocarriers was size dependant with optimal size requirement of ~25 nm or <50 nm. Thus, two copies of PEG along with a molecular weight of 20 kDa PEG appears to be a prerequisite for optimum macrophage targeting.

Support: NIH AI33789

The Application of Early Bactericidal Activity (EBA) studies in Assessing Antituberculosis Drugs

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Background: Alternative therapeutic regimens with new faster-acting drugs are needed to stop the spread of tuberculosis (TB). Quantitative and reproducible testing methods are vital to formulate appropriate treatments. Studies of the early bactericidal activity (EBA) were established as an initial step to assess new antituberculosis drugs. It measures the ability of individual antimicrobials to kill bacilli in cavities of patients with pulmonary TB at the beginning of therapy. Objectives: To retrospectively review the value of EBA studies to determine: 1) whether a drug has activity against bacilli in cavities excreted in sputum. 2) The dose size that is just ineffective and the maximum dose above which no improvement in EBA is found (therapeutic margins).

Methods: Of ten treatment-naïve patients, with smear-positive pulmonary TB were randomly allocated to standard daily doses of first line drugs for 2 to 5 days with either: isoniazid (H); rifampicin (R) pyrazinamide (Z); ethambutol (E) and HRZ in combination. Kidney showed much lower concentration of unmetabolized drug, indicating reduced nephrotoxicity with the developed formulations.

Conclusions: Thus lipid nanocarriers have high potential for the delivery of primaquine to liver with enhanced antimalarial efficacy and reduced toxicity.

Associative learning induces selective changes in the quantitative distribution of GAT-1, a high-affinity γ-aminobutyric acid transporter, in adult mouse barrel cortex

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Background: The increase of functional cortical representation of vibrissae in the mouse brain occurs as a result of classical conditioning when stimulation of selected vibrissae (CS, conditioned stimulus) is coupled with aversive reinforcement (UCS, unconditioned stimulus). The plastic change was demonstrated by labeling with antibodies against GAT-1, the γ-aminobutyric acid transporter, in adult mouse barrel cortex. We have also shown that functional reorganization of barrel cortex is accompanied by increased density of small GABAergic cells. GAD67 mRNA, and GAD67-positive puncta in the hollows of barrels of the ‘trained row’. The aim of this study was to determine whether GAT-1, a high affinity, GABA plasma membrane transporter is affected by learning.

Methods: Unbiased optical dissector counting was applied to sections from the mouse barrel cortex that had been immunostained using a polyclonal antibody against GAT-1 when the trained row was stained with standard avidin-biotin complex (ABC) method. Quantification of numerical density of GAT-1 positive puncta was performed for “trained” (CS+UCS n=5), and control groups; which received only stimulation of vibrissae without the unconditioned stimulus (CS n=5) and naïve mice (n=5).

Results: One of the most important findings of the experiment is that learning (CS+UCS) induced increase in numerical density of GAT-1 puncta in hollow barrels of trained row of vibrissae (the experimental side value was on the average 50% higher compared to the control side). In contrast, numerical density of GAT-1 puncta was unchanged in both control groups.

Conclusions: 1) Present data suggested that GAT-1 may be involved in learning-dependent changes in layer IV of the barrel cortex. 2) In any event, these changes in the immunolabeling for GAT-1 in hollow barrels of trained row of vibrissae would cause an increased uptake of GABA. (Supported by MNiSW grant 188).

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
Potential use of denosumab in the treatment of giant cell tumor of bone

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Background: Giant cell tumor (GCT) of bone, or osteoclastoma, is usually a benign but locally aggressive osteolytic neoplasm in which monocytic macrophage/osteoclast precursor cells and multinucleated osteoclast-like giant cells infiltrate the tumor. While the origin of GCT is unknown, the tumor cells of GCT produce chemotactic factors that can attract osteoclasts and multinucleated osteoclast precursors, such as receptor activator of NF-κB ligand (RANKL). RANKL is over-expressed in thestromal-like tumor cells of GCT, and is a key mediator of osteoclast formation and survival that could recruit osteoclast-like giant cells to the tumor. The potential role for tumor cell-osteoclast interaction/oстеoclast dependence, as in a paracrine loop in which the osteoclast-like cells support the stromal tumor cells, in GCT is unknown. New agents that inhibit osteoclastogenesis via the RANK/RANKL pathway, such as denosumab (a fully human monoclonal antibody against RANKL), may be useful in the treatment of GCT.

Methods: Gene expression in 8 GCT was done by microarray using the Affy U133 chip set. A multi-center open-label phase II trial, of patients with GCT treated with denosumab, 120 mg monthly, with loading doses on days 8 and 15 of month 1 was reviewed. The primary endpoint was tumor response (elimination of ≥95% of giant cells or no radiographic progression of the target lesion) at week 25. In an interim report, 24 patients had received denosumab and 15 were evaluable for efficacy.

Results: Gene expression suggested an osteoclastogenic environment in GCT, with RANKL mRNA expression highest. In ongoing trials, 13 of 15 evaluable patients showed positive response: 9 of 9 had a histologic response, and 4 of 6 had a radiographic response. The 2 patients who did not meet radiographic response criteria had stable disease per investigators. Clinical benefit was observed in 9 patients, including reduced pain and increased range of motion. No treatment-related serious adverse events or neutralizing anti-denosumab antibodies were reported.

Conclusions: In the interim analyses reported to date of a multi-center phase II trial, 13/15 evaluable patients with recurrent or unresectable GCT responded to denosumab treatment. The results suggest a possible trophic effect of the recruited osteoclasts on the primary tumor cells. Further investigation of denosumab as a new therapy for GCT is warranted.

Genetic aspects of tramadol PK and PD

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Background: Tramadol, widely used analogues, is mainly metabolized into an active metabolite O-demethyltramadol (M1) via polymorphic CYP2D6. Aim of our studies were to objectify PK properties and opioid action of tramadol in healthy volunteers over 24 h post-dose and drug–induced miosis over 12 h post dose. Tramadol and M1 analysis were done by GC-MS method.

Methods: Three studies with healthy volunteers (n=87), and one study with patients after knee arthroscopy (n=100) were conducted. Volunteers received a standard dose of 100 mg twice daily, and blood samples were drawn from healthy volunteers over 24 h post-dose and from patients after knee surgery. Samples for PK analysis were collected from healthy volunteers over 24 h post-dose and drug – induced miosis over 12 h post dose. Tramadol and M1 analysis were done by GC-MS method. Pupillography was performed using monocular infrared pupillograph "Pupilscan EHRLICH II” in a quiet and fully darkened room. Visual analogue scale with verbal description, drug consumption, and necessity to use emergency pain treatment. For each subject, three lymphocyte cultures were set up according to the protocol of the present study to determine whether nitrofurantoin and furagin, used for long-term prophylaxis of pyelonephritis (UTI), may induce chromosome aberrations (CAS) and sister chromatid exchanges (SCEs) in lymphocytes of the treated patients.

Results: Chromosome Damage in Peripheral Lymphocytes of Children with Urinary Tract Infection after Antimicrobial Therapy with Nitroheterocyclic Compounds

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Background: Nitroheterocyclic compounds such as nitrofurantoin [N-(5-nitro-2-furfurylidene)-1-aminohydantoin] and furagin [N-(5-nitro-2-furyl-allylidene)-1-aminohydantoin] have been used effectively in the therapy of urinary tract infection (UTI) for many years. Since recurrences of UTI is common in susceptible individuals, the objective of the present study was to determine whether nitrofurantoin and casagin, used for long-term prophylaxis of pyelonephritis (UTI), may induce chromosome aberrations (CAS) and sister chromatid exchanges (SCEs) in lymphocytes of the treated patients.

Methods: Cytogenetic analysis was performed in 153 blood samples from 109 children aged from 0.2 to 13 years and suffering from UTI. The treatment consisted of either local administration of nitrofurantoin or furagin at doses of 5-8 mg/kg/day for the first 7 days and at doses of 1-2 mg/kg/day for the rest of the treatment period. Blood was sampled before the start of the therapy and after 1, 3, 6 and 12 months of the therapy. For each subject, three lymphocyte cultures were set up according to conventional techniques. CAS were scored in 100 first mitotic division cells and SCEs in 25-50 second division cells per individual.

Results: CAS frequency was not affected by age, nitrofurantoin or furagin therapy or previous treatment with antibiotics. The only effect on CAS was a higher frequency of CAS in controls (mainly acetoxy) due to uncontrolled reproduction that preceded the therapy. Analysis of different types of CAS revealed two statistically significant trends: decrease in the frequency of acetoxy (Yr) with the time elapsed since uncontrolled reproduction (Xr) (Yr=-1.783.0.062Xr ^2−0.77), and increase of chromatid exchanges (Yr) with duration of furagin therapy (Xr)=(Yr=0.065.0.19Xr ^2−0.83). SCE frequency was not affected by age, antibiotic therapy and nitrofurantoin or furagin therapy. A time-independent significant increase in SCE frequency was found in lymphocytes of children treated with furagin.

Conclusions: 1) Furagin induced SCEs and CAS (chromatid exchanges) in lymphocytes of the treated patients. Nitrofurantoin was not genotoxic. 2) Since both nitrofurantoin and furagin are interchangeable in their antimicrobial efficiency, preferential use of nitrofurantoin in treatment of UTIs might be recommended.
Background. Primary cutaneous lymphomas comprise a heterogeneous group of lymphoproliferative disorders characterized by clonal accumulation of T-lymphocytes (CTCL) or B-lymphocytes (CBCL) homing to the skin. While a variety of registered treatments, including immunotherapies exist for CTCL, no registered therapy is available for CBCL, apart from radiotherapy and surgery. For the management of relapsing CBCL, after first line radiotherapy or surgery, various off-label therapeutic options are being explored. Presently all current treatments including radiotherapy and surgery do not cure the disease but lead to relapses at various frequencies, indicating further medical need for alternative therapeutics. Systematic administration of recombinant IFN-γ has shown promising response rates in CTCL, however, therapy with recombinant cytokines is limited by their short half-life and significant side effects upon systemic administration. Transgene SA is developing TG1042 (Ad5/IFN-γ) which is based on a replication-deficient adenovirus type 5 (Ad5) carrying the human IFN-γ gene. TG1042’s anti-tumoral activity relies on the anti-proliferative, immunomodulating and anti-angiogenic activities of the transgene-encoded IFN-γ cytokine.

Methods. The investigational treatment consists of intraläsional injections of TG1042 at a dose of 5 x 10^11 viral particles (vp) per lesion up to six lesions at days 1, 8, 15 of a monthly treatment cycle. Patients are treated for up to 4 cycles. An open-label, multicenter, dose-escalation TG1042/01 phase I/II trial enrolled 39 patients with advanced cutaneous T-cell lymphoma (CTCL) and multilesional cutaneous B-cell lymphoma (CBCL). Intraläsional injections of TG1042 were included as a dose escalation regimen in the phase I part. Treatment with TG1042 induced a high rate of local clinical responses: 19 (57%) of 33 evaluable patients responded to the treatment with 9 complete responses (CR) and 10 partial responses (PR). All six enrolled CBCL patients responded to the TG1042 treatment (100% response). Concerning safety, the results from this phase I trial demonstrated that TG1042 was well tolerated up to the highest dose level (3 x 10^12 vp). Adverse events (AEs) were mostly of grade 1 and grade 2. The most common reported AEs were injection site reactions, fever, chills, and fatigue. In a multicenter, open-label TG1042/02 phase II study of intraläsional administration of TG1042 in relapsing CBCL patients (PCMZL, PCFCL), it is now enrolling patients at centers in Switzerland, France, USA, Serbia, Croatia, and Poland. This is the first clinical trial ever performed in CBCL with a large cohort of 41 patients. Twelve patients are currently enrolled in the study. Interim results of the ongoing phase II study will be presented.

Conclusions. Adenovirus-based cytokine transfer may represent a new treatment modality for cutaneous lymphomas and especially for CBCL given the limited number of treatment options and the lack of registered drugs for this indication. TG1042 may become a convenient treatment option for patients who prefer other treatment options than radiotherapy or surgery. The cytostatic treatment effect on nucleoli of leukaemia cells at the single cell level

**SMETANA K**

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Background: Nucleoli are multifunctional organelles. Their structure, cytochemistry and size reflect main cell states as proliferation, differentiation, resting state, ageing and death.

A multitudinous number of nucleolar molecules, including peripheral shell. They are in “resting” cells and reflect the reversible state of the molecular machinery that mediate causes and cures of psychic depression. Neuroreceptors that may mediate causes and cures of psychic depression. PET radiotracer has suitable properties for neuroimaging of antidepressant binding sites in the brain of laboratory animals and humans. The receptor occupancy information


**SMITH DF**

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Background: Dr. Paul Ehrlich knew the importance of a close match between spatial features of drugs and biomolecules, and his vision continues to guide research in medicinal chemistry. Today, positron emission tomography (PET) is a powerful imaging tool used during drug development to explore the inner workings of the brain.

**RESULTS:** Our research has focused in recent years on the antidepressant compound mirtazapine, radiolabelled with C-11 for PET. PET radiotracer has suitable properties for neuroimaging of antidepressant binding sites in the brain of laboratory animals and humans. The receptor occupancy information obtained by therapeutic doses of mirtazapine can be quantified by [C11]mirtazapine in regions of the living human brain. Studies of the enantiomers of [C11]mirtazapine in vivo and in vitro showed differences in the stereoselectivity of the findings.

**Conclusion:** [C11]Mirtazapine provides a reliable PET-procedure for quantifying receptor sites involved in antidepressant actions.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts
Resolution of cancer and infection induced wounding is an essential factor in immune enhancement. The answers to why and how revealed

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Background: The lifecycle of Cancer can be simplified into two phases of the disease: abnormal growth is a prerequisite for carcinogenesis and the later phase, malignancy, where abnormal growth cannot continue without harnessing the body's wound response. Induced wounding in the stroma provides many benefits to Cancer progression including destruction and remodeling of competing healthy tissue, growth of new blood vessels and immune suppression.

Methods: Systems Analysis of published journal literature

Results: Evidence shows that Cancer induced wounding is critically dependent upon up-regulation of the Angiotensin type 1 (AT1R) receptor. Classically associated with the vascular system, expression of AT1R is a systemic cellular response to oxidative, hypoxic and physical stresses. Activation of AT1R by Angiotensin II leads to the production of a host of pro-inflammatory mediators including cytokines, chemokines, adhesion molecules and other factors such as TGF-beta, VEGF and Matrix Metalloproteinases. Early clinical studies utilising Angiotensin Receptor Blockade in Cancer and other chronic inflammatory diseases (many of which having, or suspected to have, an infectious component) demonstrate increased patient survival, reduced biological distortion and pain.

Conclusions: Lessons learnt from a systems analysis of the behaviour of Angiotensin in Cancer explains that the induced 'Wound that never heals' is an environment that is of vital importance in suppressing the adaptive immune response and that Cancers and other infections utilise chronic inflammation in order to spread and evade learned immune responses. Angiotensin Receptor blockade synergises with conventional and development drug targets and agents that could work in combination with ARBs to treat most diseases.

Rapid intervention: the role of antivirals in containing or controlling pandemic influenza

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The continuing spread of the influenza A(H5N1) virus among poultry in Southeast Asia, Europe, the Middle East and Africa represents the most serious risk of a human pandemic in decades. Although amounts of pre-pandemic vaccines, will be available at the start of a pandemic, the principal interventions are likely to be antivirals and social distancing. Modelling data suggest that antiviral treatment of infected individuals and post-exposure prophylaxis (PEP) of household contacts, combined with containment, could have immediate effects during a pandemic.

The oral antiviral agent oseltamivir (Tamiflu) is active against multiple influenza strains and is the only neuraminidase inhibitor to have shown activity in the clinical management of patients infected with highly pathogenic H5N1. Oseltamivir is the only antiviral that is strongly recommended by WHO for the treatment of individuals with confirmed or strongly suspected H5N1 infections and PEP of high-risk exposure groups. WHO has recently highlighted that early treatment with oseltamivir is recommended, and data from uncontrolled clinical trials suggest that it improves survival, underscoring the need for rapid medical intervention.

OSeltamivir phosphate is available for use in all age groups, is well tolerated and is associated with few adverse events. Early treatment is likely to have the greatest impact on pandemic influenza.

Activation of Ancient Stress-resistance Pathways by Molecular Triggers, Age and Disease Intervention

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Background: Not only the genetic blueprint, but also the ability to access survival pathways by epigenetic signaling determine species survival. The pathways, though present and thought to last, may only require an activation trigger of latent protective mechanisms. Molecular mimics of the environmental cues are engaged as triggers of the protective and rejuvenating molecular pathways from the studies with this laboratory and from numerous other investigators. Hibernation is the environmental response to stress. Delta opioids are used to tolerate hemorrhagic stress in our published studies.

Objectives: To assess the effect of one such agent to tolerate ischemic stress of hemorrhage, deltorphins (D, Delt-Davir) a mimic of hibernation factor, our following experiments were preformed.

Methods: Rats were fitted with femoral arterial and venous catheters for measurements of mean arterial pressure (MAP), heart rate (HR), and intravenous injections of isoflurane, saline, or 2 mg/kg of D-Davir. Hemorrhage, 30% (5 mL) of total blood volume was collected from the arterial catheter. MAP-HR was fitted to a logistic equation to determine baroreceptor reflex sensitivity. 2) Improved MAP, and this recovery may involve a change in baroreflex sensitivity.

Results: This comparative analysis between RTA and PAP pointed to a partitioning of the virus with the RTA-RNA complex, the anti-inflammatory properties.

Conclusion(s): Protein engineering can be used to increase stability and solubility of recombinant subunits proteins enabling increase soluble expression from heterologous host systems and higher production yields during manufacturing while maintaining antigenic properties and eliminating undesirable features.

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All abstracts are listed in alphabetical order of the presenting author.
NOVEL MODULAR TRANSPORTERS WHICH SIGNIFICANTLY ENHANCE EFFICACY OF TRANSPORTED DRUGS AND IMPART CELL SPECIFICITY TO THEM

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Background: A major challenge in the development of specific and effective cancer treatments is the fact that exploiting a molecular target that is accessible (e.g. cell membrane or extracellular matrix) is critical for achieving tumor selectivity while delivering the therapeutic to the cell nucleus is generally required for maximizing the therapeutic effect. An intriguing approach to this conundrum is to utilize a hybrid molecule to achieve both goals by linking together modules with different functionalities.

Methods: We have employed recombinant technology to develop targeted therapeutics that include modules for addressed delivery both to tumor cells and into compartments within these cells that are the most sensitive to the drug. Our modular nanotransports (NT) are polypeptides possessing (a) an internalizable ligand module providing for target cell recognition and subsequent receptor-mediated endocytosis of the NT by the cell; (b) an endosomolytic module ensuring escape of the NT from the endosomes; (c) a module containing a nuclear localization sequence, thereby enabling translocation of the NT into the cell nucleus; and (d) a carrier molecule for attachment of a drug.

Results: We produced different NTs containing different ligand modules enabling recognition and internalization of the NTs by the following target cancer cells: melanoma, glioma, epidermoid carcinoma, acute myeloid leukemia and neuroblastoma. Cytotoxic efficacy of either -emitting radionuclides or photodynamic agents transported by the NTs turned out to be 10-3000 times higher than that of free drugs. Moreover, the NTs impart cell specificity to the drugs; e.g., free doxorubicin imparts no selective toxicity to target and non-target cells, whereas if they are attached to the NT they become highly phototoxic for non-target cells at the concentrations that were phototoxic for target cancer cells.

Conclusions: Cell specificity and high efficacy of many therapeutics can be achieved with the use of modular NTs with preset properties, which would ensure recognition of the desired target cell and subsequent directed transport to the subcellular compartment of choice.

LC-MS/MS SHOTGUN PROTEOQUIMICS OF LUNG CANCER PLEURAL EFFUSIONS IDENTIFIES THE PROGNOSTICALLY RELEVANT EPITHELIAL-TO-MESENCHYMAL TRANSITION PROTEIN PERIODIN

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Background: Malignant pleural effusion of advanced lung cancer is a valid source for detection of secreted N-glycosylated biomarker proteins (N-GP), because tumor cells grow during weeks in this liquid. Therefore, such N-GP effusion profiles for detection of secreted N-glycosylated biomarker proteins (N-GP) are potentially used to target other tumor cells. Free photosensitizers are equally phototoxic for target and non-target cells, whereas if they are attached to the NT they become highly phototoxic for non-target cells at the concentrations that were phototoxic for target cancer cells.

Methods: Malignant pleural effusions of 5 patients with lung adenocarcinoma and 5 non-malignant controls were used for triplicate N-GP capturing by solid-phase extraction. After trypsin digestion and PNGase F release, liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) was performed. For biomarker protein validation, a tumor tissue microarray of 533 patients with surgically resected non-small cell lung cancer (NSCLC) was analysed by immunohistochemistry.

Results: In the total of 10 effusion samples, 170 non-redundant proteins were detected with probability of 0.9 to 1. The specificity for the N-Glycolyt was 88%. Mass spectrometric penetration into the moderate to low protein concentration range (mikro-nanogram/mL) occurred. The EMT protein peridotin was confidently identified in several malignant effusions. Of the 533 NSCLC patients, 48% had squamous cell carcinoma, 47% adenocarcinoma and 5% adeno-squamous carcinoma. High protein expression of peridotin in either peripheral desmoplastic stroma or in tumors, increased in a proportionally scored by two pathologists, correlated with male gender, higher stage, higher pT category and larger tumor size; and in only stroma with tumor relapse (all p-values <0.05). Further, high stromal expression was found to be a prognostic factor for decreased progression-free survival on univariate analysis (p-value 0.007).

Conclusions: Pleural effusion is a useful biomarker source for lung cancer. Reduction of sample complexity by N-GP capturing allows detection of low abundance biomarker proteins. The EMT protein peridotin is closely associated with advanced disease and may thus be integrated in progression models of NSCLC.

TREATMENT OF AGITATION IN DEMENTIA: PHARMACOLOGY FOR SYMPTOM RELIEF FOR THE MAGIC BULLET

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Background: Few controlled studies are available to guide the clinician in treating potentially assaulative elderly individuals with psychiatric disorders. This is an important concern, with up to 90% of dementia patients suffering from agitation during its course. Safety and tolerability are the cornerstones for the use of buproprion and antipsychotic medications, making anticonvulsants an attractive alternative. With no one medication as a magic bullet, the side effects profile of a given medication may be used to its advantage in treating a patient’s unique target symptoms. In other cases, targeted dosing of several medications concomitantly for specific symptom relief may yield the most effective results. Each medication has side effects that may be concomitant in this paradigm.

Methods: We reviewed the current research on the efficacy, safety and tolerability of anticonvulsant medications used for individuals over 60 with agitation due to dementia. Recommendations for use under specific circumstances are made, depending on the specific symptoms of the patient.

Results: Gabapentin, useful in patients with co-existing pain, may cause ataxia. Oxcarbazepine, with few efficacy studies, is associated with severe hyponatremia. Carbamazepine, important in the treatment of bipolar disorder and pain disorders, may cause hyponatremia and pancytopenia. Tipiramate may be helpful in the weight gain commonly induced by other medications such as the commonly prescribed antipsychotic drugs, but is associated with significant cognitive impairment. Valproate, also used for pain syndromes may cause transaminase elevation, pancreatitis, and hyponatremia.

Conclusions: With no medication approved for the treatment of agitation associated with dementia, the clinician has a dilemma in nonetheless needing to treat the out of control yet frail patient. There is no one class of medication that is most effective or best tolerated for this vulnerable population. Better outcomes often are achieved by combining different medications to optimize efficacy while limiting adverse effects. Anticonvulsants are an example of a medication class that may be used to advantage in this way; either to augment the effect of other drugs for therapeutic effects, or to diminish side effects.
Targeted Pulmonary Delivery of Aerosolized PGE1: A “Magic Bullet” for Neonatal Pulmonary Hypertension?

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Background: Extensive experience with inhaled nitric oxide in neonatal persistent pulmonary hypertension (PPHN) shows lack of improvement in 30-40% of patients. Inhaled PGE1 (IPGE1) is a potential selective pulmonary vasodilator. Our aim is to characterize the stability, emitted dose (ED) and the aerodynamic particle size distribution (APS). Manufacturer’s specifications are not available. An additional goal is to evaluate pulmonary drug delivery and toxicity.

Methods: Chemical stability and ED of PGE1 solution were evaluated during jet nebulization in a neonatal conventional (CMV) and high frequency (HFV) ventilator circuit by High Performance Liquid Chromatography - Mass Spectrometry. A six-stage cascade impactor was used to determine APS. Pulmonary deposition was assessed with MRI in seven ventilated piglets using T1 weighted spin-echo sequence. Toxicity was evaluated in ten ventilated piglets receiving either high dose IPGE1 or nebulized saline continuously for 24 hours.

Results: There was no significant degradation of PGE1 following nebulization. The ED of PGE1 was 32-40% during CMV and 0.1% during HFV. The PGE1 aerosol had a mass median aerodynamic diameter (MMAD) of 1.4 μm and geometric standard deviation of 2.9 with 90% of particles being < 4.0 μm in size. On MRI, a significant increase in signal intensity (SI) was observed in the lungs 10 min after start of aerosol. At the end of 90 min, the SI increased by 98%. There was no evidence of adverse cardiac-respiratory effects related to IPGE1 in ventilated piglets. Histomorphological changes included moderate to severe focal ulceration, flattening of the bronchial epithelium and loss of cilia in the trachea and bronchi.

Conclusions: 1) Nebulization of PGE1 during neonatal CMV or HFV is efficient and results in rapid nebulization without altering the chemical structure. 2) On the basis of the low MMAD, and large proportion (93%) of particles ≤0.15μm, one can predict predominantly alveolar deposition. 3) This was validated on MRI, with evidence of effective pulmonary aerosol delivery within 10 minutes of contrast nebulization. 4) Inhalation of high dose IPGE1 was not associated with adverse cardiopulmonary effects and produced minimal signs of pulmonary toxicity even after 24 hours. 5) Thus, IPGE1 may be a safe and effective selective pulmonary vasodilator in PPHN.

Oxidative stress in periodontal diseases and associated neoplasias: a role for antioxidants?

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Background: Studies indicate that plaque-associated inflammatory loading in periodontal diseases leading to tooth loss may have associations with developing cancer. There is documentation of a significant association between cancer of the lung, kidney, pancreas, haematological cancers and periodontal diseases. The development of oxidative stress with malignant progression of a tumour has been reported, with evidence of effectiveness of antioxidants as anti-cancer agents. For example, dehydroascorbic acid reacts with homocysteine thiocysteine found in cancer cells resulting in the formation of the toxic compound 3-mercaptopyrrolinodisulphide which kills cancer cells. Other effective agents reported in this context are green tea polyphenols, melatonin and vitamin D also reported to be beneficial in periodontal disease outcome. Green tea polyphenols are able to induce apoptosis in various tumour cell systems. This apoptotic mechanism has been shown to be targeted at mitochondria and executed by caspase 3. This has been demonstrated by subjecting tumour cells which had either a caspase 3 deletion or expression of wild type caspase 3 to increasing concentrations of green tea polyphenols indicating mitochondrial targeting. The development of oxidative stress with malignant progression of a tumour has been reported. Drug resistant cell populations can emerge in response to a milieu of oxidative stress. Cellular adaptation is likely to be multifactorial, coordinating factors that induce hypoxia, nuclear factor kappaB (NF-kappaB) and their targets downstream that are linked to resistance mechanisms. This resistance can be overcome by treating the cells with NO mimetic agents to restore their sensitivity to cytotoxic agents both in vivo and in vitro. Mechanisms involved include vascular changes, tumour oxygenation and antioxidant effects, down regulation of the glutathione detoxification / redox buffering system, inhibition of key transcription factors and DNA repair systems.

Conclusions: It is relevant that periodontal diseases are associated with a small but significant overall cancer risk which persists in non-smokers. Periodontal disease may be a useful marker of a susceptible immune system, or directly affect cancer risk as a result of inflammatory loading. Formulation of effective therapeutic dosing of agents shown to have efficacy in this context is a challenge.

All abstracts are listed in alphabetical order of the presenting author.
Betulonic acid and its alamine amide derivatives - a new multi-target agent for tumor chemotherapy

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Background: A multi-target agents based on natural pentacyclic triterpenoid platforms present a new approach to drug design. They may be use to both prevent and therapy of cancer as well as to be synergistic with standard anti-cancer treatments. Aims: 1) To development a multi-target agents using betulonic acid (BA) as new triterpenoid platform. 2) To study in vivo theirs anti-oxidant, anti-inflammatory, antitumor and antimetastasis activities. 3) To study the effect of agents on potency and tissues damage of standard chemotherapy.

Methods: This study included 80 male mice CBA, 220 female mice C57Bl/6, 160 outbred male mice. Transplantable tumors were Lewis lung carcinoma (LLC) and mice RLS lymphoma. Agents were administered orally at single (250, 500 mg/kg) or daily (50 mg/kg/day during 7 or 14 days) doses. A standard poly-chemistry ACOP (adriamycin, cyclophosphamide, Oncovin, prednisone) was carried out 24 hours before dosing. It was estimated: tumor growth, volume areas of metastasis, dystrophic and necrotic changes in some tissues (morphometry using light microscopy), a thio-barbituric acid-reactive substances in serum, anti-inflammatory activity (histamine- and carrageenane-induced mice paw edema models).

Results: It was synthesized 4 derivatives BA with $\delta$-ala or $\delta$-alaa substitution at C-28 and their methyl esters. All agents at single dose reduce tumor growth (by 10-20%) and inhibit metastasis LLC (by 40-50% as to control). All agents possess anti-oxidant (40-60%) and anti-inflammatory (30%) activities (P<0.05), after 7 days of dosing (50 mg/kg/day) only two agents: MPT and its $\delta$-ala analogues significantly decreases LLC metastasis in lung (by 9 times, P<0.05) and RLS metastasis in kidneys (by 1.7 times). Treatment of mice with these agents ACOP leads to rise its antitumor (by 1.3 times, P<0.05) and antimetastasis (up to 2 times, P<0.05) potency. The acid derivative with $\delta$-ala decreased a number of metastasis ACOP, (P<0.05) and increases a dystrophic changes (to 28%) in the liver and kidneys. The agent induces a glycogen synthesis in the liver.

Conclusions: Among alamine amide derivatives BA it is found an agent that has high antimetastasis activity, rises a poly-chemistry potency and decreases tissues damage.

Metalloporphyrins are Versatile and Powerful Therapeutics: Biomimetics of SOD, Peroxyredoxin, and cyt P450

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Background: Mn-porphyrins with proper substituents possess high superoxide and peroxynitrite scavenging activity, and can modulate redox-based cellular signaling pathways thereby exerting protection in radiation, cancer, diabetes, Alzheimer disease, and ischemia-reperfusion injury. Few Mn–porphyrins and their methyl esters are efficacious cyt P450 mimics, while Zn-porphyrins are potent photo sensitizers.

Mimicking cyt P450

Methods: We compared different Fe- and Mn-porphyrins in their efficacy to catalyze hydroxylation (activation) of cyclophosphamide (CP) to 4-OH-CP, the active cytotoxic metabolite, under biologically relevant conditions. Thus 1 mM CP was incubated with 10 Mn-porphyrins in the presence of 2 mM ascorbic acid in PBS at pH 7.4 and 5.5 at 37 °C under aerobic conditions (0.26 mM O2).

Results: The most effective Fe-P450-like catalyst was the highly electron-deficient Fe(III) mesotetras(2,6-difluoro-3-sulfonatophenyl)porphyrin (4.6% yield). All porphyrins were more effective at pH 5.5 than at pH 7.4.

Conclusions: Fe-porphyrin can hydroxylate (activate) cyt P450 at similar efficiency as cyt P450 does (2.6% yield). The observed pH effect could be of importance in terms of specificity since tumor extracellular pH was reported to be as low as 5.2 and near the surface of macrophages to be as low as 3.6.

Antioxidant potential:

MnTnHex-2-PyP is a lipophilic (hexa-) analogue of MnTe-2-PyP (ethyl-, highly potent SOD mimetic) which we tested for its efficacy in stroke model: 5 min after and during reperfusion of middle cerebral artery occlusion, rats were given 75 or 225 mg/kg MnTnHex-2-PyP. It was estimated: tumor growth, volume areas of metastasis, right/Left SPV ratio mean in depression w as 0.77 $<0.05$) and RLS metastasis in kidneys (by 1.7 times). Treatment of mice with this agent increases antioxidant (40-60%) and anti-inflammatory (30%) activities (P<0.05).

Conclusions: We found fluctuations of vestibular dysfunction depending on mood state in Bipolar Disorder patients. Left vestibular activity was the parameter that varied the most between mood states, for this reason we suggest that the stabilizing effect of antidepressor the urgent need for innovative therapeutics.

Inter-kingdom cell-to-cell inhibitors: a novel target for antimicrobial drugs

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Background: The worldwide challenge of antimicrobial resistance and the paucity of novel antibiotics underscores the urgent need for innovative therapeutics. The increasing understanding of bacterial pathogenesis and inter-cellular communication, when combined with contemporary drug discovery tools and technologies, provides a powerful platform for translating basic science into therapeutic applications to combat bacterial infections. Inter-kingdom chemical signaling bridges the communication between bacteria and their hosts. Many bacterial pathogens rely on a conserved membrane sensor, QseC, to sense and respond to host adrenergic signaling molecules and to bacterial signals to promote expression of virulence factors.

Methods: We used a combination of high throughput screen of small molecules, qRT-PCR, and virulence tests to identify QseC inhibitors.

Results: Here we show that small molecule inhibitors of QseC-mediated activation markedly inhibit the virulence of several pathogens in vitro and in vivo in animal models. We identified a potent small molecule, LED209, which inhibits binding of signals to QseC, preventing QseC’s autophosphorylation, and consequently inhibiting QseC-mediated activation of virulent gene expression in enterohemorrhagic E.coli (EHEC), Salmonella typhimurium and Francisella tularensis. LED209 also prevented formation of lesions on epithelial cells by EHEC, and F.tularensis survival within macrophages. Remarkably, LED209 treatment protected mice from lethal S.typhimurium and F.tularensis infection. LED209 is not toxic and does not initiate pathogen growth.

Conclusions: Inhibition of microbial virulence without inhibition of growth may engender less selective pressure to promote the generation of resistance. As demonstrated herein, inhibition of inter-kingdom inter-cellular signaling constitutes a novel and highly effective strategy for the development of a new generation of broad spectrum antimicrobial agents.
Penetration of Bioactive Proteins and Peptides across Stratiﬁed Mucous in a Porcine Ex-Vivo Model

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Background: The non-keratinized stratiﬁed mucosa lining the oral cavity, esophagus and vagina provide relatively permeable surfaces to deliver therapeutic agents to treat local and systemic diseases. On the other hand, if micro-organisms and their toxins present at these surfaces penetrates the tissues they can lead to cross infection particularly in one case where we believe probiotics will be of use both as a cleaning disinfectant material and as a soap. My presentation will highlight areas where we can make possible improvements to contain the development and spread of microbial resistance both in the community and in hospitals. How use in selected cases of probiotics can be beneﬁcial in this pathological conditions ranging from periodontal disease to toxic shock syndrome and their toxins present at these surfaces penetrate the tissues they can lead to pathological conditions ranging from periodontal disease to toxic shock syndrome and addiction. This study examined the use of a porcine ex-vivo model to study mucosal permeability and tissue pathogenesis.

Methods: Pig mucosal tissue provides an excellent model for human for drug delivery and permeability studies (Squier C.A. et al., J Pharm Sci. 97:9-21, 2008). Specimens of pig buccal and vaginal mucosa were removed at slaughter and placed in thermostatted continuous ﬂow mucosal perfusion chambers for up to 12 hours (Squier C.A., et al. J Pharm Sci. 86:82-84. 1997). Our studies examined the ﬂux of a bioactive peptide, 111I labeled transforming growth factor β (TGFβ) across buccal mucosa in the presence of phosphate buffered saline (PBS) or the mucoadhesive polymer, chitosan. In separate experiments, the ﬂux of 51Cr labeled staphylococcal toxic shock syndrome toxin (STST-1) across vaginal mucosal tissue was determined in keratinocyte serum-free medium (KFSM) medium alone or with the addition of 50 µg/m of a staphylococcal virulence factor, α-hemolysin. The ﬂux of TGFβ was signiﬁcantly (p<0.05) increased in the presence of chitosan (24x96 dpm/cm²/min) compared to PBS (36x18 dpm/cm²/min) suggesting that the mucoadhesive may provide higher local concentration of compound at the mucosal surface than does a solution. The ﬂux of TGFβ across the vaginal mucosa in the presence of α-hemolysin (2.7±0.8) was signiﬁcantly (p<0.05) greater than that in medium alone (1.3±0.4 ng/cm²/min) and was associated with marked histological changes in the epithelium, including massive lymphocytic inﬁltration and separation of epithelium from connective tissue, suggesting a synergistic role for virulence factors in the penetration of bacterial toxins.

Conclusions: The use of porcine tissue ex-vivo provides a realistic model for studying the kinetics and pathogenesis of tissue permeation by bioactive peptides and pathogenic toxins in real time for up to 12 hours.

Signiﬁcant ﬁnancial support: This work was supported by an unrestricted grant from the Procter and Gamble Company, Cincinnati, OH.
Acetylsalicylic Acid: an Immune-Modulator in HIV Infection

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Background: Management of AIDS in a country where more than 80% of population lives in deep poverty is a challenge, which no clinician would like to experience. To begin with lack of water and sanitation, not to mention nutritional deficit. Antiretrovirals are an important part of ART regimen against HIV and the problem of drug resistance, especially with the use of Acetylsalicylic Acid as an immune modulator in HIV infected patients.  

Methods: We randomized 20 naive HIV-positive men and women into placebo and 1.2g of Acetylsalicylic Acid. All participants were supported by nutritional program with three meals per day. Baseline CD4 count, viral load (VL), and the SIV sequences by testing recombinant gvpC-SIV genes will support the biosynthesis of chimeric GvpC fusion proteins and generate functional organelles. Results: Each of the three different exogenous DNA sequences had been stably retained in the recombinant gvpC gene. The transformations with each of the selected recombinant plasmids was successful and had conferred a functional Vacα status. Antisera from mice immunized with r-GV and also monkey anti-SIV sera each recognize the chimeric r-GvpC protein.

Conclusions: 1) Different recombinant gvpC-SIV genes will support the biosynthesis of chimeric GvpC fusion proteins and generate functional organelles. 2) Monkey antibody elicited by in vivo infection with SIV recognizes these expressed SIV sequences as SIV peptides. 3) Test of antisemum elicited by immunizing mice with r-GV demonstrated notable and long term antibody titers. Continued presence of elevated antigen specific antibody is a feature critical to immunization based protection. The observed level of humoral responses and the maintenance of elevated responses to encoded peptides are consistent with the suggestion that in vivo there may be natural and slow release of epitopes over time.

Rabies in Bats and Protective Treatments of Humans in Europe

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Background: Surveillance of rabies in bats across Europe during the last 50 years revealed rabies infection in bats in many European countries and also some human cases after exposure to rabid bats. Humans should be treated against rabies pre-exposure and post-exposure. There is a need to protect some professions and cavers well. According to WHO, rabies control needs to be adapted on the rabies surveillance in each country and rabies pre-exposure and post-exposure treatment of humans needs to assume bat rabies as well. Goals: 1) Find out if post-exposure vaccination against rabies is implemented in particular countries; 2) Find out if preventive treatments of bat researchers and cavers are used as well.  

Methods: Questionnaire was dispatched by mail to contributors of the Rabies Bulletin Europe from European countries. We asked questions concerning recommendation for post-exposure treatment after a bat bite and for pre-exposure treatment of bat researchers and cavers.

Results: 15 countries across Europe participated in the study: Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Iceland, Netherlands, Norway, Poland, Serbia, Slovenia, Switzerland and United Kingdom. Post-exposure treatment is more frequently practiced (13 of 15 countries) than pre-exposure treatment of bat researchers (11 of 15 countries involved in the study). Pre-exposure treatment of cavers is practiced only in Czech Republic, France and Israel.

Conclusions: 1) Distribution of European lyssa viruses in Europe is not well understood because only few countries have suitable monitoring of these infections; 2) Growing knowledge concerning bat habitats and availability of successful treatment with rabies vaccine and immunoglobulin is interesting to medical doctors, veterinarians, cavers and bat researchers; 3) Reasons for actualizing recommendation concerning treatment against bat rabies are the changing world with large tourist movement and investigation of epidemiological importance of emerging infectious diseases as well.
New Medicines, New Problems: Understanding the Failure of the Phase I Clinical Trial of Tgn1412

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In 2006 a near-fatal “cytokine storm” occurred in six healthy volunteers during the phase-I clinical trial of TGN1412, a therapeutic superagonistic CD28-specific monoclonal antibody, signalling a failure of pre-clinical safety testing. Subsequently we established that TGN1412 could stimulate a “cytokine storm” in vitro but only if correctly presented to human cells by immobilisation onto plastic or when added in the presence of endothelial cells. These novel procedures would have predicted the toxicity of this superagonist and are now being applied to emerging immunotherapeutics and to other therapeutics that have the potential to act upon the immune system. Data from these novel in vitro procedures suggests that the dose of TGN1412 given to human volunteers was close to the maximum immunostimulatory dose. In contrast to humans we found that TGN1412 is not superagonist for cells from the non-human primate model used for safety testing. A “cytokine storm” was not seen during pre-clinical in vivo safety testing as non-superagonist CD28-specific monoclonal antibody is not pro-inflammatory for human or non-human primate cells.

Cytokine storm and therapeutic strategies

A “cytokine storm” was not seen during pre-clinical in vivo safety testing as non-immunostimulatory dose. In contrast to humans we found that TGN1412 is not superagonist for cells from the non-human primate model used for safety testing. A “cytokine storm” was not seen during pre-clinical in vivo safety testing as non-superagonist CD28-specific monoclonal antibody is not pro-inflammatory for human or non-human primate cells.

Subsequently we established that TGN1412 could stimulate a “cytokine storm” in vitro but only if correctly presented to human cells by immobilisation onto plastic or when added in the presence of endothelial cells. These novel procedures would have predicted the toxicity of this superagonist and are now being applied to emerging immunotherapeutics and to other therapeutics that have the potential to act upon the immune system. Data from these novel in vitro procedures suggests that the dose of TGN1412 given to human volunteers was close to the maximum immunostimulatory dose. In contrast to humans we found that TGN1412 is not superagonist for cells from the non-human primate model used for safety testing. A “cytokine storm” was not seen during pre-clinical in vivo safety testing as non-superagonist CD28-specific monoclonal antibody is not pro-inflammatory for human or non-human primate cells.

Conclusions: 1) The decreased threshold for spontaneous SR Ca2+ release induced by DPc10 is likely to be proarrhythmic and therefore consistent with the disease phenotype. 2) The absence of effect of DPc15-mut can be explained if the disease mutation impairs the ability of the peptide to interact competitively with region 1. 3) The ability to mimic the effects of disease mutations in normal cells using targeted peptides provides an opportunity for the development of novel therapeutic strategies.

Type 2 diabetes mellitus is a major health problem in the 21st century. New therapies are needed to control metabolic abnormalities, and also to preserve β-cell mass and to prevent loss of β-cell function. Glucagon-like peptide 1 (GLP-1) is a drug candidate which potentially fulfils these conditions. Mice lacking dipeptidyl peptide IV are protected against obesity and insulin resistance. Several DPP-4 inhibitors are in clinical development; these are orally active and increase levels of active GLP-1, which in turn increases insulin secretion and reduces glucagon secretion and thereby lowers glucose levels. Most experience exists for sitagliptin and vildagliptin, which both have a long duration of action, allowing once-daily administration. DPP-4 inhibition is an efficient treatment of type 2 diabetes, both as monotherapy and combination therapy. Because of its efficiency, safety, and tolerability in association with the oral mode of administration, it is expected that DPP-4 inhibition will be a first-line treatment of the early stage of type 2 diabetes, particularly in combination with metformin or thiazolidinediones. The DPP-4 inhibitor vildagliptin improves insulin sensitivity and β-cell function, leading to improved postprandial glycemia in subjects with IFG, who are known to have β-cell dysfunction. Thus, vildagliptin may prevent progression to diabetes in high-risk subjects. Incretin mimetics are a new class of pharmacological agents with multiple antihyperglycemic actions that mimic several of the actions of incretin hormones. Exenatide is the first incretin mimetic approved for clinical use. Exenatide therapy can be considered as an alternative to insulin in patients with treatment failure on metformin monotherapy or on metformin together with a sulfonylurea.

Conclusions: 1) The long-term consequences of DPP-4 inhibition on β-cell function and the durability of glucose lowering achieved with sustained DPP-4 inhibition require careful clinical assessment. It seems prudent to pursue additional detailed studies of the biological role(s) of DPP-4 and the consequences and safety of high selective DPP-4 inhibition in experimental and clinical models of diabetes. Therapy for diabetes will probably not alter radically in the next few years unless long-term data demonstrate other advantages over metformin and insulin. However, agents modulating GLP-1 are likely to be playing a major role in combating the world-wide burden of diabetes in the 21st century.
Staphylococcal Enterotoxin Vaccines: Future Utility for Fighting Staphylococcus aureus?

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Background: There is little doubt that Staphylococcus aureus is as much today a microbial menace as it was when first discovered by Alexander Ogston in the late 19th century. With rising rates of antibiotic resistance evident now in the 21st century, it seems clear that additional measures must be made to counter S. aureus. Past efforts of pacifying this pathogen and its myriad virulence factors have included the generation of recombinant-attenuated versions of staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin 1 (TSST-1). Naturally, these toxins are superantigens that bind to major histocompatibility complex II and specific T-cell receptors. These protein toxins stimulate T cells that appear less relevant with the probable exception of ABCB1 in adult patients. ABCB3 (MRP3) has a strong prognostic impact in AML and ALL independent of age group. However, ABCB3 does not cause drug resistance in vitro. Therefore, it remains to be elucidated whether its correlation with poor response to therapy is causative or just an epiphenomenon. Specific inhibitors of ABC-transporters can sensitize leukemic cells to chemotherapy. For some types of leukemia it would be desirable to develop drugs that inhibit a set of ABC-transporters.

Transdermal Delivery of Naltrexone, In Vitro Testing to Human Volunteers

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Background: Alcoholism and opiate addiction lead to major health-related problems and societal costs for people around the world. Therefore, research and development for improved pharmacologic treatments for drug and alcohol abuse are very important. Naltrexone, an opioid antagonist, is currently used in oral tablet form to help maintain opioid addicts in a drug-free state. Most recently, naltrexone has been indicated as an adjunct in the treatment of alcohol dependence, as well as to reported to reduce alcohol craving in certain alcoholic populations. Transdermal delivery of naltrexone is desirable for opioid addicts and alcoholics in order to help reduce side effects associated with oral therapy and improve compliance. Naltrexone itself does not have the essential physicochemical properties that would allow a therapeutic dose of the drug to cross the human skin barrier. Methods: For the last 12 years we have been designing and synthesizing produgs, which are more skin permeable than naltrexone, in order to make a therapeutically successful drug delivery system. We have hypothesized that produgs of naltrexone and produgs in combination with micronedule treatment will improve the transdermal delivery rate of naltrexone. These produgs have made excellent research tools for investigating quantitative structure-permeability relationships (QSPr) for transdermal flux and optimization of flux in combination with micronedule enhancement. Results: These produgs/micronedules have improved naltrexone delivery rates across the skin because of optimized physicochemical properties for faster diffusion. Testing has been completed to measure the drugs penetration and concurrent bioconversion through human skin in vitro with and without micronedule use, and to examine the pharmacokinetics of the drugs in guinea pigs in vivo with and without micronedule use. Correlation of our in vitro data with the in vivo model allowed us to design and complete a human proof-of-concept study using micronedules and naltrexone hydrochloride. Conclusions: We have been able to improve the flux of naltrexone across the skin up to 10-fold with these methods. The current focus of our studies is to increase the rate of naltrexone transdermal delivery by combining micronedule technology with a produrg and formulation approach. Funding NIHROI 6DA13425.

Which ABC-transporters should we target in leukemia?

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ABC-transporters are a large family of proteins involved in active transport across biological membranes. Some members of this family cause drug resistance in malignant diseases via ATP dependent drug efflux from malignant cells. This phenomenon was intensively analyzed in leukemia. ABCB1 (P-g) and ABCG2 (BCRP) were shown to be associated with poor response to chemotherapy in adult acute myeloid leukemia (AML). Both proteins confer resistance to a wide range of chemotherapeutic drugs in vitro. Therefore, they represent possible therapeutic targets. In pediatric AML, this is the case for ABCG2 but not for ABCB1. In acute lymphoblastic leukemia (ALL), both proteins appear less relevant with the probable exception of ABCB1 in adult patients. ABCG2 or ABC transporters generally are not inducible in various other malignancies while the ABCB1 is inducible in melanoma, bladder cancer, and prostate cancer, respectively. Therefore, it is important to study the expression profiles of ABCG2 and ABCB1 in the context of leukemia. In addition, the use of specific inhibitors, like valinomycin for ABCB1 or ketoconazole for ABCG2 can help to evaluate the contribution of these proteins to drug resistance. Results: Valinomycin was shown to be an effective inhibitor of ABCB1 in AML and ALL, respectively, while ketoconazole was less effective. Conclusion(s): The results of these studies will provide additional tools against ABC-transporter mediated drug resistance in leukemia.
Background: The prognosis for metastatic melanoma remains poor even with traditional adjuvant or interferon therapy. 5-year survival is markedly higher amongst patients undergoing metastasectomy. Unfortunately not all are suitable for metastasectomy. Alternative agents for systemic therapy have, to date, offered no greater rates of survival beyond traditional therapy. A toll-like receptor 9 agonist, PF-3512676 (formerly known as CPG 7909) is currently being evaluated for its potential.

Case presentation: We present the case of a 54-year-old Caucasian male with completely resected metastatic cutaneous melanoma after immunotherapy. The patient initially progressed during adjuvant high-dose interferon, with metastases to the liver, spleen, and pelvic lymph nodes. During an 18-month treatment period with PF-3512676 (formerly known as CPG 7909), a synthetic cytosine-phosphorothioate-guanine rich oligodeoxyribonucleotide, slow radiologic disease progression was demonstrated at the original disease sites. Subsequent excision of splenic and pelvic nodal metastases was performed, followed by resection of the liver metastases. Histologic examination of both hepatic and splenic melanoma metastases showed extensive necrosis. Subsequent disease-free status was demonstrated by serial positron emission tomography (PET).

Conclusions: Existing evidence from phase III trials suggest systemic treatment with PF-3512676 is capable of provoking a strong tumor-specific immune response and may account for the prolonged tumor control in this instance.

Ocular Infections: Impact of Resistance to Fluoroquinolones and Other Antibiotics on Topical Therapies

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Background: Ocular infections of the anterior segment include bacterial conjunctivitis and keratitis. Both of these infections are treated with topical antibiotic drops. Ophthalmic antibiotic drops include aminoglycosides, tetracyclines, fluoroquinolones, fusidic acid, and most recently in the last 15 years, fluoroquinolones (FQs). An important question is whether or not acquired resistance to an antibiotic limits the efficacy of the corresponding ophthalmic products.

Methods: The clinical and microbiological efficacy was correlated with in vitro susceptibility of the pre-therapy pathogens in Alcon-sponsored clinical studies with FQs conducted during the past decade.

Results: Clinical and microbiological efficacy results from more than 3,000 conjunctivitis patients revealed that frequency of treatment failures due to a specific pathogen were directly proportional to the frequency of occurrence of the specific pre-therapy bacterial species. Generally, the pre-therapy pathogens from treatment failure patients were susceptible to FQs and did not change their FQ susceptibility as a result of FQ therapy.

Conclusions: Antibiotics applied topically that are rapidly bactericidal lead to more rapid resolution of clinical signs and symptoms and eradication of pathogens that are bacteriostatic. FQ ophthalmic products have high concentrations (3000 – 5000 mcg/ml) relative to the FQ MICs for pathogens, even those that have become resistant to FQs. Treatment failures due to FQ resistant pathogens did not occur more frequently than treatment failures due to FQ susceptible pathogens indicating equivalent efficacy for FQ susceptible and resistant pathogens. Moreover, in no case did the FQ susceptibility of a pre-therapy pathogen change as a result of FQ therapy.
Fluoride and Aluminum Interactions: AlFx as the Magic Bullets Producing Aberration of G Proteins

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Background: The wide use of fluoride (F) and aluminum (Al³⁺) in medicine, industry, and agriculture, started the era of supplementation of living environment with these ions as never before. Many effects primarily attributed to F are caused by its interactions with Al³⁺. The effects of aluminum fluoride complexes (AlFx) - new analogues of phosphate groups - have been demonstrated by many studies with proteins, intact cells, and whole animals. The widespread use of AlFx as a general activator of G proteins provided evidence that AlFx represent Magic Bullets for human body. The warning of the potential danger from the interactions of Al³⁺ and F to human health has emerged from research on cell signaling.

Methods: Biochemical and immunohistochemical methods were used for in vitro experiments. Meta-analysis techniques for human studies were used because only summary statistics are typically available in the literature.

Results: Fluoride affects the activity of numerous enzymes and various biological processes. The effective dose is much lower in the presence of trace amounts of Al³⁺. AlFx interact with all known G protein-activated effector enzymes. Numerous findings demonstrate the positive correlation with the impairment of homeostasis, growth, development, cognition, and behavior. The predictability from in vitro data as well as the correlation with numerous epidemiological, ecological, and clinical studies from fluoridated communities will be presented. AlFx may induce most of the pathological hallmarks of autism and Alzheimer's disease.

Conclusion: 1) The definition of a safe concentration of F for humans must consider that the dose is much lower in the presence of Al³⁺. 2) It is evident that AlFx is a molecule giving the false information, which is amplified by processes of signal transmission. Pharmacologists estimate that up to 60% of all medicines used today exert their effects through G protein signaling pathways. AlFx are made Magic Bullets that produce aberration of G proteins. 3) Assessment of the health risks linked to the F and Al³⁺ interactions would contribute significantly to the decline of many several disorders in the 21st century.

Supported by MSM 0021620806


Inhibitory Effects of Demecolcine on NK Cell Functions: Implications for Cancer Therapy

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Background: The cytostatic property of the microtubule-disrupting agent demecolcine has previously been exploited to some degree in oncology. Neoplasms of NK cells are generally highly aggressive. However, current therapies (chemotherapy and/or radiotherapy e.g.) are suboptimal and overall survival is poor. Effects of demecolcine on NK cell functions were studied using the aggressive NK leukemia cell line KHYG-1 as a model.

Methods: KHYG-1 was treated with different demecolcine doses, 0.1 to 30 μM, for 15 hrs at 37 °C with 5% CO₂ washed 3-4X in PBS and compared to untreated cells in subsequent experiments. Micromotubules (anti α-tubulin) and cytotoxic granules (anti perforin) were imaged by confocal microscopy. Viability, Annexin V and 7AAD staining, and receptor expression levels were analyzed by flow cytometry and cytokicity measured in a flow cytometric assay. Degranulation (anti perforin) after target contact and phosphorylation of the kinase ERK were assessed by immunoblotting.

Results: In KHYG-1 cytolytic granules are constitutively polarized, normally a consequence of tumor target contact. Demecolcine treatment (10 μM) dematurated granules reversibly in about 90% cells. Cytotoxicity was significantly reduced at doses mentioned above, although, not completely abrogated. This was likely due to re-polarization of granules during assay duration as assessed in parallel imaging experiments. About 20-40% KHYG-1 were positive for Annexin V and about 10-20% positive for 7AAD at 0.1 to 30 μM doses. Degranulation after target contact was inhibited, expression of important activation receptors, NKG2D and NKG4, down-modulated to about 60%, and ERK phosphorylation to about 45%. In all experiments, a plateau effect at doses ≥1 μM was noted (Suck, G. et al. Int Immunol 2008, 16:1347-54).

Conclusions: Demecolcine significantly inhibited KHYG-1 functions and could be envisaged as a treatment for NK neoplasms, with the potential to induce apoptosis, interfere with cytotoxicity and proliferation as a cytostatic drug. A potential drawback is the reversibility of its effects. Combination therapy with the proteasome inhibitor Bortezomib, which has recently been shown to induce apoptosis in malignant NK cell lines, followed by hematopoietic stem cell transplant, is envisaged to improve this approach.
Recombinant therapeutic proteins - - from the viewpoint of a biotechnologist
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Recombinant proteins are possible magic bullets for pharmacological interventions of the various diseases. However, it is challenging to find a protein with a desirable effect. Moreover, it is also tough to produce a recombinant protein of interest in a form achieving full activity. We have explored pharmacologically relevant proteins using recombinant technologies. Protein C is an anti-coagulant protein requiring gamma-carboxylation of the Gla domain for its activity. To ensure its sufficient modification we needed to produce recombinant protein C in mammalian cells. The culture conditions must be optimized to accomplish the satisfactory level of its productivity with enough gamma-carboxylation. Now protein C is on the market as an anti-septic agent from Eli Lilly.

Peroxidin was first identified as an osteoblast specific factor-2 from cultured osteoblastic cells with expectation that it should exhibit the activity to stimulate osteoblast cell growth. In order to seek its pharmacological activity toward bone we produced peroxidin in a high yield by the use of a baculovirus expression vector system. Recently peroxidin gains much attention due to its potential to treat patients with heart diseases despite the expectation at its discovery. Thus, the multidisciplinary expertise is required for the development of recombinant magic bullets.

### Some Uses of Metal-Based Complexes as Anti-Tumor Agents

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**Background:** Gold(III) complexes have long been known to exhibit anti-cancer properties. However, their medicinal application has been hampered by their poor stability in solutions. In this study, we aim at 1) preparing a variety type of physiologically stable gold(III) complexes; 2) studying the unprecedented structure-activity relationship for stable gold(III) complexes; 3) identifying potential anti-cancer drug hit(s) according to their in vitro and in vivo anti-cancer and anti-angiogenic activities; and 4) illustrating their anti-cancer mechanism(s).

**Methods:** By using strong σ-donor dianionic porphyrinato ligands, we developed a family of gold(III) porphyrin complexes having different hydrophilic and hydrophobic substituents. Their IC50 values toward different panels of cancer cell lines were determined by MTT assay, and some of their in vivo anti-cancer activities were studied by using Blattia rats and nude mice models. To elucidate the potent anti-cancer mechanism(s), DNA array, proteomics, western blotting and computational docking analyses have been employed.

**Results:** The IC50 values of the gold(III) porphyrin complexes were found to correlate with their lipophility and their cellular uptake. Two gold(III) porphyrins, namely gold-1a and gold-2a, exhibited tremendous in vitro cytotoxicity with IC50 = 50 nM toward some nasopharyngeal carcinoma cell lines. In vivo studies showed that these two complexes could prolong survival of the HCC-bearing rats and/or significantly inhibit tumor growth in nude mouse models. Western blotting and computational docking analyses revealed that some anti-angiogenic proteins such as Bcl-2 and Mcl-1 are important cellular targets. A gold(III) porphyrin with saccharide conjugation (gold-3a) was found to be relatively not cytotoxic, but exhibits significant cytostatic and anti-angiogenic activities on MS1 cells.

**Conclusions:** The enhanced stabilization of the gold(III) ion and the ease of its structural modification render porphyrin ligands to be advantages in the development of physiologically stable, bioactive gold(III) porphyrin complexes with potent anti-cancer and anti-angiogenic activities.

**Plasmodium Heme Crystallization: Methylene Blue to the Quinolines**

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**Background:** Attempting to make quinine, WH Perkins created the aniline dye mauve, which provided the rational basis of staining microorganisms for inhibition. Ehrlich started from the aniline dye, methylene blue, to discover the first synthetic antimarial proven effective in humans. Ehrlich later launched the first drug screen to identify compound 806 for syphilis. Methylene blue and the quinolines like chloroquine both bind heme and the heme crystal, hemozoin, to effectively kill Plasmodium parasites. Aims: 1) to show the mechanism of intracellular heme crystal formation and inhibition by methylene blue and the quinolines and 2) to compare inhibition of both P. falciparum and heme crystal inhibition by more than 2,000 existing drugs.

**Methods:** This study utilized subcellular fractionation, electron microscopy, mass spectrometry and heme crystallization assays to demonstrate heme crystal formation in Plasmodium. Different heme crystallization inhibition assays were compared to P. falciparum culture inhibition by the Johns Hopkins Clinical Compound Library, comprised of over 2000 approved drug molecules.

**Results:** P. falciparum intracellular heme crystals were observed to grow within neutral lipid nanospheres by electron microscopy. Subcellular fractionation followed by mass spectrometry identified principally neutral lipids closely associated to heme crystals. The neutral lipids promoted efficient heme crystallization, inhibitable by the quinolines and methylene blue. The clinical compound drug screen inhibition of parasite and heme crystal crystallization identified novel antimalarial activity of the antifolate, aminopterin, and the epipodophyllotoxin, teniposide.

**Conclusions:** Dye or quinoline binding to heme and heme crystals effectively kills malaria parasites. Screening an approved drug library for malaria or other diseases is potentially an efficient means to discover novel activities of existing drugs which can rapidly be translated into human clinical trials.

**Personalized Medication with Estramustine Phosphate for Advanced Prostate Cancer after Screening of the CYPIA1 gene polymorphisms**

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**Background:** Estramustine phosphate (EP) is a chemoendocrine agent applied for the treatment of advanced and/or hormone refractory prostate cancer. Gastrointestinal (GI) toxicity is observed frequently during EP therapy in prostate cancer patients. Formerly, we reported that polymorphisms in the CYPIA1 gene were significantly associated with GI toxicity during EP therapy. Aim of this study is to determine whether screening of the CYPIA1 gene polymorphisms is a useful method to achieve personalized medication with EP for advanced prostate cancer.

**Methods:** After screening of the CYPIA1 gene polymorphisms, a total of 39 patients with advanced or hormone refractory prostate cancer was regarded as a low-risk group for GI toxicity with EP. The methods of genotyping assay were a TaqMan assay or a direct-sequencing method. All of patients were administered EP 280 mg/day orally and assessed their toxicities monthly. Three of 39 patients withdrew EP therapy within 8 weeks because of other toxicities (liver dysfunction, lung edema, and gynecomastia), and they were excluded from the analysis. Formerly, we experienced EP therapy with same regimen for 55 patients of advanced prostate cancer without screening of the CYPIA1 gene polymorphisms. We compared the incidence of GI toxicity according to the NIH Common Terminology Criteria for Adverse Events v3.0 between former study and present one, and evaluated the efficacy of the gene screening. The Ethics Committee of the University of Tokyo approved this study.

**Results:** Follow-up period of present study was 557 ± 254.6 (means SD) days. Incidence of GI toxicity of former study and present one was 40.0% (22/55) and 19.4% (9/36), respectively. The odds ratio was 0.36 (95% CI, 0.13 - 0.94; P = 0.036). In the multivariate analysis, the hazard ratio was 0.21 (95% CI, 0.06 - 0.65; P = 0.006). The withdrawal of EP therapy because of GI toxicity in former study and present one was 18.2% (10/55) and 2.8% (1/36), respectively. Conclusions: Screening of the CYPIA1 gene polymorphisms prior to EP therapy could achieve a personalized medication with EP for the patients with advanced prostate cancer.
Background: Ca\(^{2+}\) is a highly versatile intracellular second messenger that regulates many complicated cellular processes, including cell proliferation, cell death, and cell adhesion. It has long been believed that in nonexcitable cells, such as hematopoietic cells, store-operated Ca\(^{2+}\) entry is the principal route of Ca\(^{2+}\) influx. Increasing evidence suggests the existence of functional L-type Ca\(^{2+}\) channels (LTCCs) in various immune cells, including T, B and natural killer cells, but their molecular entity and functions are poorly understood. We have previously reported that in mast cells IgE plus antigen (IgE/antigen) stimulates a dihydropyridine (DHP)-sensitive Ca\(^{2+}\) channel that is immunologically and pharmacologically related to LTCCs. In the present study we attempted to reveal the molecular entity of this Ca\(^{2+}\) channel. Moreover, since mast cells do not undergo apoptosis upon IgE receptor activation, while the Ca\(^{2+}\)-ATPase inhibitor thapsigargin (Tg), which cannot activate the LTCC-related Ca\(^{2+}\) channel, causes sizably apoptosis, we elucidated the potential role of the Ca\(^{2+}\) channel in mast cell survival.

Methods: The expression of LTCCs mRNA was evaluated using RT-PCR analysis. The expression of LTCCs on the cell surface was determined by flow cytometry. Apoptotic cell death and overall cell death were evaluated by annexin V/propidium iodide double-staining, cytochrome c leakage and the membrane potential collapse.

Results: The mast cell line rat basophilic leukemia (RBL-2H3) expressed the \(\alpha\)-subunit mRNA and express the \(\alpha\)-protein on their surface. The cells underwent apoptosis upon IgE receptor activation, while the Ca\(^{2+}\)-ATPase inhibitor thapsigargin (Tg), which cannot activate the LTCC-related Ca\(^{2+}\) channel, causes sizably apoptosis, we elucidated the potential role of the Ca\(^{2+}\) channel in mast cell survival.

Conclusions: LTCCs play a critical role in the survival of mast cells and potentially other immune cell types which express them through maintaining mitochondrial Ca\(^{2+}\) homeostasis and preventing mitochondrial integrity disruption.

Antimalarial drug resistance in Indonesia: A molecular analysis

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Background: Malaria remains a major public health problem in Indonesia, causing approximately 30 million clinical cases and several thousands deaths annually. The endemic malaria in Indonesia is caused mainly by P. vivax, P. falciparum and P. malariae. Resistance to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) have started to emerge in Indonesia. The P. falciparum isolates from Lombok region were found to express a number of drug resistance markers. The molecular basis of their resistance to CQ and Sp/Pm has been studied using PCR-based methods.

Methods: Analysis of the pfcrt, pfmdr1, pfdhfr and pfdhps genes and their orthologues in P. vivax, that were employed Polymerase Chain Reaction (PCR), restriction fragment length polymorphism (RFLP), and partial DNA sequencing. The sequence analysis was done by DnaStar software.

Results: Analysis of drug resistance markers revealed that most of the isolates are resistant to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP). The PfCRT 76-Val phenotype was detected in 94% of the isolates with PfMDR1 86-Thr phenotype in 91% of the isolates. The PfMQR 440X was detected in 90% of the isolates. The PfDHFR 515N was detected in 98% of the isolates, and the PfDHP 590G was detected in 92% of the isolates.

Conclusions: The molecular basis of chloroquine and sulfadoxine-pyrimethamine resistance in P. falciparum from Lombok region was analyzed using PCR-based methods. The results indicate that the isolates are resistant to chloroquine and sulfadoxine-pyrimethamine due to PfCRT 76-Val and PfMDR1 86-Thr phenotypes, respectively.
Advent of Pharmacology of Nociceptors Initiated by Capsaicin Has Opened a New World in Neurohumoral Regulation

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Background: Capsaicin, the hot principle of chili is the first magic bullet for nociceptors. Its receptor cloned in 1997 is a thermosensor ion gated by noxious stimuli, named Transient Receptor Potential Vanilloid 1 (TRPV1). Furthermore, substance P and other neuropeptides released from the activated capsaicin-sensitive nerve endings eliciting – without axon reflexes – neurogenic inflammation and other effenter tissue responses. We also described that released somatostatin inhibits inflammation at distant sites.

Methods: 1. Mechanical, noxious heat thresholds of rat paw was measured in hyperalgesia (plantar incision, heat injury, etc.) with and without stimulation of the acutely denervated contralateral paw. 2. Neurogenic inflammation evoked by electrical stimulation (dorsal roots, nerves) or by irritants was similarly tested after subtracting some of the other parts of the body (paw, eye, vagal nerve). 3. Sensory neuropeptides were measured by radioimmumunoassay. 4. Gene deleted mice (TRPV1, sst4R) were also used.

Results: 1. Contralateral counter-irritation (capsaicin, mustard oil) diminished the hyperalgesia by more than 50%. Antagonists of somatostatin (cyclosomatostatin) or cannabinoid CB1 receptor counteracted these effects. 2. Antidromic stimulation (dorsal root, sciatic nerve) enhanced the plasma somatostatin level over 3-fold and inhibited by around 50% inflammation evoked by capsaicin, carrageenin or contralateral dorsal root stimulation. The inhibition was absent in rats pretreated with somatostatin antibody or after perineural capsaicin pretreatment. 3. 0.1 Hz contralateral dorsal root stimulation. The inhibition was absent in rats pretreated with TRPV1, sst4R receptor antagonists are in Phase II clinical trials.

Conclusion: Capsaicin-sensitive major subsets of sensory neurons subserve unorthodox local effector and systemic antinociceptive, antiinflammatory “sensocrine” functions. Drug candidates of TRPV1 antagonists or somatostatin receptor agonists are in Phase II clinical trials.

Role of testosterone and estrogen in the regulation of bone metabolism in men and women

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Sex steroids (testosterone, 17 -estradiol) are major regulators of bone metabolism in men and women. After menopause, dramatic decrease in the sex hormones results in an increased bone resorption. Antiestroids (tamoxifen) and aromatization of androgens may inhibit bone loss.

Methods: Bone density was measured in the lumbar spine and the total body by dual-energy X-ray absorptiometry (DXA) in a dose-ranging study with oral administration of tamoxifen (0.5 mg for 4 weeks and 1 mg for 4 weeks). Osteocalcin, receptor activator of nuclear factor κB ligand (RANKL), Wnt/β-catenin, and cathepsin K were measured in serum and bone biopsy samples. The bone mass was assessed at the proximal femur and lumbar spine.

Results: Significant increase in bone density was observed in the lumbar spine (p<0.01) and the total body (p<0.05). There was no significant change in the serum osteocalcin, RANKL, and cathepsin K levels. Wnt/β-catenin was significantly increased in the bone biopsy samples from the lumbar spine (p<0.05).

Conclusion: Tamoxifen is effective in inhibiting bone loss in men and women. Further studies are required to determine the mechanisms of action of tamoxifen in the regulation of bone metabolism.

EHR LICII – 2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich
Nürnberg, October 3-5, 2008

Treating the Untreatable: Alpha 1-Adrenoreceptor Antagonist Prazosin for PTSD, Disruptive Agitation in AD and Alcohol Dependence

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Background: Although the CNS noradrenergic system has been implicated in multiple neuropsychiatric disorders, the postsynaptic alpha 1-adrenoreceptor (alpha 1-AR) has been addressed, as a pharmacotherapeutic target only in cardiovascular and urologic disorders. Clinical and neuropathologic studies suggest that increased responsiveness to norepinephrine (NE) at the alpha 1-AR contributes to the pathophysiology of post-traumatic stress disorder (PTSD), trauma nightmares, disruptive agitation in Alzheimer’s disease (AD) and alcohol dependence. Prazosin, a CNS active alpha 1-AR antagonist, was evaluated for efficacy and tolerability in these three difficult to treat disorders.

Methods: Study 1: 40 Vietnam war veterans with PTSD and intractable nightmares were randomized to prazosin (achieved mean bedtime dose = 5 mg) or placebo. 3 weeks of titration and 8 weeks of mean dose. Study 2: 22 elderly AD patients with treatment resistant disruptive agitation were randomized to titrated prazosin (achieved daily dose 2 mg tid and 3 mg bedtime) or placebo. 2 weeks of titration and 4 weeks of mean dose. Study 3: 17 men seeking abstinence for alcohol dependence were randomized to titrated prazosin (achieved mean daily dose = 4 mg tid and 8 mg bedtime). 2 weeks of titration and 4 weeks of mean dose.

Results: Prazosin was significantly and substantially superior to placebo for: (study 1) reducing nightmares and improving sleep and overall clinical status in PTSD; (study 2) reducing disruptive agitation by Neuropsychiatric Index (NPI) and Brief Psychiatric Rating Scale (BPRS) scores in AD; and (study 3) reducing alcohol ingestion and days drinking in persons seeking abstinence.

Conclusion: Prazosin appears effective for three different neuropsychiatric disorders that have been very difficult to treat. Larger and longer placebo controlled trials of prazosin are underway for all three conditions. This data introduces the novel use of prazosin, an alpha 1-AR antagonist, for several different neuropsychiatric disorders that have been very difficult to treat.

Effects of neurotoxic and neuroprotective compounds on cholinergic neurons are mediated by alterations in acetyl-CoA metabolism

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Background: The preferential loss of septal cholinergic neurons is a main cause of cognitive deficits in course of Alzheimer’s disease (AD) and other encephalopathies of advanced age. High susceptibility of cholinergic neurons to neurodegeneration might result from the fact that they utilize acetyl-CoA, not only for energy production but also for acetylcholine (ACh) synthesis. Therefore, acetyl-CoA metabolism is a likely target for both cholinotoxic insults as well as for therapeutic approaches.

Methods: Cholinergic septum-derived SN569 neuroblastoma cells were cultured in Dulbecco’s Eagle’s medium for 2 days with subsequent treatment with neurotoxic orantioxidant compounds.

Results: The differential expression of SN569 cells by radioimmunoassay, NGF or cAMP-mediated signals, caused the induction of choline acetyltransferase (ChAT) activity, ACh synthesis/quantal release and cytoplasmatic acetyl-CoA content along with decrease in acetyl-CoA synthesis and its levels in neuronal mitochondria. The shortage of acetyl-CoA in mitochondria of differentiated cells caused their greater than nondifferentiated ones susceptibility to recognized AD pathogens such as amyloid-beta, NO, Al and Zn. These compounds caused dose-dependent increase of nonviable cell fraction and cytoplasmatic cytochrome c levels, decreases in mitochondrial enzymatic and ChAT activities, intranuclear and cytoplasmatic acetyl-CoA levels. Although the former is positively correlated with alterations in cytoplasmatic acetyl-CoA levels (r=0.96, p<0.002).

Conclusions: alpha 1-AR agonist Prazosin for PTSD, Disruptive Agitation in AD and Alcohol Dependence. These data indicate the existence of cholinergic neurons two functionally independent pools of mitochondrial and cytoplasmatic acetyl-CoA, that under pathologic conditions affect their viability and expression of cholinergic phenotype, respectively.

Authors’ disclosure statement: Supported by MNISW projects 2P05A 110 30.

All abstracts are listed in alphabetical order of the presenting author.

Abstracts

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Titanium-Based Magic Bullets Against Renal-Cell Cancer

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Background: Titaniumocene dichloride (CP2TiCl2) shows significant activity against a variety of human cancer cell lines, overcomes the resistance against platinum drugs and reached two clinical Phase II studies against renal cell and metastatic breast cancer, where CP2TiCl2 failed due to a lack in anti-proliferative activity. The aim of our research is to synthesise suitably substituted titanocene complexes protected the liver from damage in the different ways, as seen in the studies of CL resulted in liver damage because of CCl4 toxicity. As reported, the composition and the biological activity of these complexes depend on the nature of the substituents. However, the PK of SAM showed that at day one the primary decrease of CL at day three may result in liver regeneration where the active enzymes were eliminated and the damaged tissue was removed. That is, MRT remained at a high level and Vd decreased. Sho-saiko-to extract or its components protected the liver from damage in the different ways, as seen in the PK parameters; e.g. baincin kept MRT at a control level but glucyrhizin decreased it.

Methods: Ansa-titanocene dichloride derivatives are obtained through reductive dimerisation of substituted aryl fulvenes with titanium dichloride, while un-bridged titanocene compounds are available from fulvenes through the addition of lithium hydride or aryl lithium (Biogenomatetic Fulvene-Derived Titanocene Anticancer Drugs, K. Strohfeld, M. Tacke, Chem. Soc. Rev., 2008, 37, 1174-1187).

Results: We investigate the cytotoxic activity of all titanocenes on LLC-PK pig kidney carcinoma cells and that of selected titanocenes on a 36 cell line panel in vitro. Results show that our most effective titanocene has a significantly higher activity than CP2TiCl2 itself. Recently, our best titanocenes have successfully finished pre-clinical animal studies (EAT, MC77, PC3, A431 and CAM-1 in mice) as well as clinical ex vivo tests involving a variety of explanted human tumors.

Conclusions: Our best titanocene derivatives are ready for clinical trials and have the potential to become the first effective chemotherapy against advanced renal-cell cancer in the nearby future.

Acknowledgment: Support is acknowledged from CESA:S, COST, HEA/SCSB and UCD.

Assay of Therapeutic Effect in Hepatitis Using Pharmacokinetics of Salicylamide: Effects of Sho-saiko-to, a traditional Kampo medicine, and Its Chemical Components in Carbon Tetrachloride Intoxicated Rats

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Background: Pharmacokinetics (PK) is a powerful tool for detecting physiological changes in vivo resulting from injury, tumors or pregnancy. Aims: The therapeutic effect of Sho-saiko-to and its chemical components, baikalin, baicalin, glycyrrhizin and glycyrrhyzic acid, were examined using daily changes in PK behavior of salicylamide (SAM) in rats with carbon tetrachloride (CCl4)-induced hepatitis.

Methods: Male Wistar rats were intraperitoneally (i.p.) injected with 0.2 ml/kg of CCl4 in 20% olive oil, and serum ALT value measured daily. A suspension of Sho-saiko-to extract was given orally at 40 mg/kg one hour before CCl4 dose; while each component solution was given i.p. at the same dose. The rats were treated daily with the reagents in the same manner. SAM was injected intravenously into CCl4-intoxicated rats at days 0 to 5 and blood samples collected for 90 min. SAM in blood samples was determined by its fluorescence. The PK of SAM were examined using the parameters; plasma clearance (CL), the mean residence time (MRT), the volume of distribution (Vd) at steady state, etc.

Results: Serum ALT value rose to a peak one day after CCl4 dose and then decreased to the control level after three days. Bacinlin (25.3%) and glycyrrhizin (19.9%) effectively suppressed the peak value of CCl4-induced hepatitis, but Sho-saiko-to extract (39.1%), baikalin (36.4%) and glycyrrhysyic acid (47.1%) were less protective. However, the PK of SAM showed that at day one the primary decrease of CL resulted in liver damage because of CCl4 toxicity, in which the metabolic enzymes were damaged but the damaged tissue remained. Thus there was delayed MRT and Vd decreased at a control level. Furthermore, the secondary decrease of CL at day three may result in liver regeneration where the active enzymes were eliminated and the damaged tissue was removed. That is, MRT remained at a high level and Vd decreased. Sho-saiko-to extract or its components protected the liver from damage in the different ways, as seen in the PK parameters; e.g. baikalin kept MRT at a control level but glucyrhizin decreased it.

Conclusions: The PK for SAM showed that liver function recovered in a bi-phasic manner by five days after CCl4 intoxication. Sho-saiko-to extract or its components effectively protected the liver against damage.

Chemotherapeutic agents for Merkel cell carcinoma (MCC) of skin – past, present and future

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Background: In the past, chemotherapy was mostly used for metastatic MCC, although a few authors tried adjuvant chemotherapy as well. At the present time, multi-modality treatments are used. This study reports the multicenter experience on management of MCC.

Methods: Patient information was obtained from medical records of 3 Canadian and 1 French institutes. Cox-proportional hazard analysis was used to find significant prognostic factors for cause-specific (CSS) and overall survivals (OS).

Results: From 1987 to 2007, 121 cases from 4 institutes were collected. Median follow-up was 21.5 (range:0.5-163.4) months. The overall rate of distant metastases was 36/121 (30%). A total of 25 patients received chemotherapy either as initial treatment (n=10) or later on at time of relapse (n=15). Patients were treated with multiple regimens at recurrence. Chemotherapeutic agents included: Etoposide (E), Cisplatin (C), Carboplatin, Cyclophosphamide (C), Adriamycin (A), Vinristine (V), Epirubicin (Epi). Iritocan, Taxotere and Topotecan. The regimens were: EP (13 patients), CAV/E (6 patients), CAV or CEpiV (5 patients), AVE or CEpiV/E (2 patients), CEP (1 patient), weekly carboplatin (1 patient), iritocan/carboplatin (1 patient), taxotere and later topotecan (1 patient). The latter were used as third and fourth line chemotherapy in the same patient, with no response. Iritocan+carboplatin produced a partial response. The patient who received weekly carboplatin as a radiosensitizer had a partial response. Cox-proportional hazard analysis showed that postoperative adjuvant radiotherapy and stage were significant prognostic factors for CSS and OS.

Conclusions: The future magic bullet for MCC will likely be radiotherapy in combination with modern chemotherapy. Future studies of MCC should focus on newer agents used for other neuroendocrine tumors, e.g., iritocan, taxotere and topotecan.

Telephone counseling of athletes abusing anabolic-androgenic steroids and the state of drugabuse in Japanese athletes

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Background: Drug abuse, most notably anabolic-androgenic steroid (AAS) use, in athletes is widespread. As a result, athletes and exercise enthusiasts who abuse these drugs are troubled by the side effects of these illicit drugs, especially AAS.

Methods: In an attempt to improve this situation, since 1993, we have counselled athletes who abuse drugs and others with questions about AAS via telephone and tabulated the results. Counseling sessions took place by telephone every Monday between 19:00-23:00 hours. The number of cases was tabulated early each year and the specific items discussed during each consultation were categorized by key words. Cases consisted of both drug abusers and athletes who did not abuse drugs and were concerned about the side effects or other various problems surrounding the use of AAS.

Results: From 1993 to 1996 there were about 50 cases yearly; thereafter, the number of consultations dropped to about 30 to 40 cases each year. In 2002, consultations with drug abusers accounted for 52.2% of all consultations compared with 46% of all consultations from 1993 to 2002. We have found that abusers of endocrine agents exist in Japan, as well as elsewhere.

Conclusions: 1) We hope these results will demonstrate the necessity of employing public institutional counseling systems for athletes who are drug abusers in Japan, similar to the successful system instituted by the Swedish National Service. 2) Furthermore some athletes and exercise enthusiasts has been recently using the website that drug abusers control in Japan. They exchange the information about the knowledge of drug abuse each other. We accessed to the website and analyzed the contents of them by key words. We would like to report the result in detail, too.
Interferon Gene Therapy against Metastatic Cancer
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Background: The interferon (IFN) is the family of one of the most potent cytokines with various biological activities. Both type I (IFN-alpha and beta) and type II (IFN-gamma) IFNs have been used as therapeutic agents for malignant tumors and hepatitis in clinic. However, the biological half-lives of IFNs are very short. One of the promising approaches to overcome this drawback may be IFN gene therapy.

Methods: The therapeutic effect of IFNs was examined using mouse models of liver or lung metastasis following gene transfer by intravenous injection of plasmid DNA vector by the hydrodynamic-based procedure (1). In order to prolong the duration of gene expression, we designed CpG-reduced (about 80%) or completely depleted vector encoding IFNs. Therapeutic effect on allergic diseases was also evaluated using NC/Nga mice, a model for human atopics dermatitis.

Results: A significant amount of IFNs was observed in the liver and blood circulation following gene expression. In the liver metastasis experiment, IFN-expressing vector showed a profound reduction of liver metastasis and a prolonged survival (2). Hydrodynamic delivery of CpG-reduced vectors resulted in more sustained production of IFNs and a better therapeutic effect against the lung metastasis (3). Moreover, a single hydrodynamic injection of completely CpG-depleted vector resulted in much more prolonged concentration of IFN-gamma over 80 days in NC/Nga mice. The correction of the imbalance of helper T lymphocyte subpopulations was shown in the mice and various symptoms associated with the allergic disease were eliminated.

Conclusions: 1) Novel CpG-reduced vectors encoding IFNs showed prolonged gene expression with G-loci strategy following intravenous injection by the hydrodynamic method. 2) Sustained IFN gene expression by the CpG-reduced vectors resulted in enhanced therapeutic effect in lung metastatic model in mice. 3) Prolonged plasma IFN concentration was also effective for the treatment of atopics dermatitis in mice. In conclusion, IFN gene therapy would be possible through optimization of design and delivery of IFN-plasmid vector.

References:

Paclitaxel works nicely with molecular targeting cancer therapy via RNA interference
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BACKGROUND: Paclitaxel (PTX; Taxol), is used to treat patients with lung, ovarian, breast, head, and neck cancer or advanced Kaposi’s sarcoma. PTX works by interfering with normal microtubule break-down vectors following division. Although it acts as a potent anti-cancer reagent, it has a major dose-limiting toxicity from bone marrow suppression. To reduce the dosage of PTX, and to minimize hematologic side effects, we designed a combination therapy based on molecular targeting (in which the target is VEGF) to work by interfering with normal microtubule breakdown during cell division. PTX works nicely in combination with molecular targeting cancer therapy, i.e., anti-PTX, a chemotherapeutic agent, in combination with a molecular targeting therapy to reduce the antigenicity, is used as a delivery carrier of siRNAs. Atelocollagen of PC-3 tumors that had been subcutaneously xenografted in nude mice. The VEGF siRNA, together with atelocollagen, significantly suppressed the growth of PC-3 tumors that had been subcutaneously xenografted in nude mice. Atelocollagen, which is derived from bovine collagen digested with pepsin to reduce the antigenicity, is used as a delivery carrier of siRNAs. Atelocollagen contributes to the stabilization of the siRNA in tumors and delivers the siRNA to tumor tissues. These inhibitory effects of VEGF siRNA were dramatically augmented by combined treatment with PTX. The combinatory effect was cytolytic, thus reducing the initial volume of the tumor. The PTX dose (12 mg/kg) used for the combinational treatment did not cause any neutropenia or liver damage. VEGF siRNA suppressed VEGF contents in tumors, leading to the inhibition of tumor angiogenesis. Surprisingly, we also observed, for the first time, that PTX itself significantly reduced VEGF expression in the tumor. The evidence supports the phenomenon that PTX inhibits tumor angiogenesis even without any evidence of the molecules involved.

DISCUSSION AND CONCLUSION: We made it possible to reduce the dosage of PTX, a chemotherapeutic agent, in combination with a molecular targeting therapy consisting of nucleic acids, especially those based on RNA. We also found that PTX works nicely in combination with molecular targeting cancer therapy, i.e., anti-angiogenesis (anti-VEGF), via RNA.

Interaction Between Local Anesthetics, Specialty Articaine, and the Sarcolipin Reticulum Ca++-Adenosine Triphosphatase
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BACKGROUND: The calcium-dependent adenosine triphosphatase (Ca++-ATPase) is a major intrinsic protein in the sarcolipin reticulum (SR) membranes from skeletal muscles. It actively transports Ca++ from the cytoplasm to the SR lumen, reducing the cytosolic Ca++ concentration to promote muscle relaxation. Local anesthetic (LA) may induce sustained muscle contraction by inhibiting Ca++-ATPase. Aims: 1) to determine the action mechanism of articaine on the Ca++-ATPase cycle; 2) to study the different LA sensitivities on skeletal muscle isoforms of the Ca++-ATPase.

METHODS: SR vesicles from rabbit skeletal muscle were obtained as in Chomel et al (1985). Ca-dependent ATPase activity was determined as in Baginski et al (1967). ATP-dependent calcium uptake and phosphorylation with inorganic phosphate (Pi) were determined with a radioscintic technique. Transient kinetics of the Ca++-ATPase active transport was analyzed by numerical simulation (Hecht et al, 1990).

RESULTS: Articaine inhibited Ca++-ATase activity, with Ki depending on Ca++ concentration: it increased from approximately 6 mM for 0.1 mM Ca++ up to a constant value around 40 mM at [Ca++] higher than 20 mM. Anesthetic also inhibited the Ca++ uptake by isolated SR vesicles (Ki=30.53±3.4mM, n=5). Articaine increased the permeability of the membrane to Ca++ and was prevented by Ca++ and Mg++. Mg++ and H+ affected the inhibitory action of articaine on the Ca pump protein. In addition, we studied the properties and inhibitory effect of several LA (unpublished results) on Ca++-ATPase. 19.31±1.87, n= 6 for ATPase activity, K(i/tdcain)=25.10±2.95, n=5 for Ca++ uptake. K(bupivacaine)= 19.30±1.12, n= 5 for Ca++-ATase activity and K(bupivacaine)= 8.12±1.81, n=5 for Ca++(uptake).

Conclusions: 1) The activating effect of Ca++ on the ATPase activity was competitively inhibited by articaine. 2) The activating effect of Mg++ on the phosphorylation of Ca++-ATPase by Pi was also inhibited by articaine. 3) Decreasing pH increased K for articaine to inhibit the Ca++-ATase activity. 4) Articaine did not affect the reaction mechanism of the cations acting as cofactors of ATP in the catalytic site. 5) K values for lidocaine and bupivacaine in masticatory muscles were lower than those reported for these LA in white fast muscle.

Crosstalk Between TNF and NGF: Potential Implications for Alzheimer's Disease and Neuroblastoma
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Background: Neuroblastoma is a pediatric tumour of the sympathetic nervous system. While early stage neuroblastoma sometimes shows spontaneous regression, advanced neuroblastoma has poor outcome and new therapeutic strategies are needed. Nerve growth factor (NGF) can induce differentiation of neuroblastomas in vitro but fails to induce their differentiation in vivo, especially neuroblastomas. To reduce the dosage of PTX, and to minimize hematologic side effects, we designed a combination therapy based on molecular targeting (in which the target is VEGF) to work by interfering with normal microtubule breakdown during cell division. PTX works nicely in combination with molecular targeting cancer therapy, i.e., anti-PTX, a chemotherapeutic agent, in combination with a molecular targeting therapy to reduce the antigenicity, is used as a delivery carrier of siRNAs. Articaine inhibits the inhibitory action of articaine on the Ca pump protein. In addition, we studied the properties and inhibitory effect of several LA (unpublished results) on Ca++-ATPase.

Methods: 1) Prolonged plasma IFN concentration was also effective for the treatment of atopics dermatitis in mice. In conclusion, IFN gene therapy would be possible through optimization of design and delivery of IFN-plasmid vector.

Results: We have reported recently that overexpression of p14ARF is not necessary for NGF-dependent differentiation of neuroblastoma cells. NGF can induce synthesis of tumour necrosis factor receptor (TNF-FNR) in neuroblastoma cells. Although TNF is a cytokine that can promote cell death through TNFR1 receptor, TNF induced by NGF activates another TNF receptor, TNFR2. When signalling through TNFR2 was inhibited, NGF induced differentiation of neuroblastoma cells without overexpression of p14ARF. Thus, signalling through TNFR2 appears to cause the insensitivity of neuroblastoma cells to NGF-dependent differentiation, rather than insufficient expression of p14ARF.

Conclusion: Our findings could provide new therapeutic strategies for neuroblastomas. Furthermore, induction of TNF in neural cells by NGF suggests a possible feedback loop of TNF and NGF expression between neurons and glial cells, since glial cells can produce TNF in response to ATP. This loop could promote survival of neural cells in inflammation. However, once their balance is disturbed, this loop could contribute to either excessive death of neural cells or excessive proliferation of immature neural cells, i.e. neurodegenerative or cancorous conditions.


**Mechanism of Fluoroquinolone resistance in Shigella and Salmonella species**

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**Background:** Enteric fever and shigellosis remain a major cause of morbidity and mortality in worldwide. Ciprofloxacin, a fluoroquinolone antimicrobial agent is generally highly effective in treating typhoid fever and shigellosis patients. We report the emergence of complete ciprofloxacin resistance in Bangladesh.

**Objectives:** The aim of the present study was to characterize and understand the molecular mechanism of fluoroquinolone resistance in Shigella flexneri and Salmonella Typhi strains recently isolated in Bangladesh.

**Methods:** A total of 1728 Shigella species isolated between 2004 and 2008 and 765 Salmonella Typhi strains isolated between 2006 and 2007 in the Clinical Microbiology Laboratory from diarrheal patients at ICCRB hospital were tested for MIC by E-test and agar dilution method in addition to antibiotic susceptibility testing following the recommendations of CLSI. Plasmid profiling, PFGE and sequencing analysis were performed in order to determine the clonal relationships and mutation analysis of QRDR of gyrA, gyrB and parC.

**Results:** Of 1728 Shigella species isolated between 2004 and 2008, 104 (6%) isolates were resistance to ciprofloxacin. Most of ciprofloxacin resistant isolates were S. flexneri 2a (80%). Resistance to ciprofloxacin of S. flexneri 2a increased from 0% in 2004 to 95% in June 2008. Of the 765 Salmonella Typhi strains, 474 (62%) strains were resistant to nalidixic acid. Of the 474 nalidixic acid resistant strains, 402 (85%) showed reduced susceptibility to ciprofloxacin. The complete resistance to ciprofloxacin increased from 2.8% in 2006 to 11 (4.5%) in July 2007. The isolates showed reduced susceptibility to ciprofloxacin to the MIC ranged from 0.064-0.25 µg/mL and the complete ciprofloxacin resistant strain ranged from 8-32 µg/mL. Sequence analysis of QRDR of ciprofloxacin resistant strains of S. flexneri and S. Typhi revealed that all had mutations in gyrA (Ser→Phe) and/or (Asp→Ain or Gln) and a single mutation in parC (Ser→Ain) whereas none of the susceptible strain had the mutation in their QRDR region. In addition, a novel mutation point was detected at outside the QRDR at position 211 (His→Ty) in gyrA gene of fluoroquinolone resistant strains of S. flexneri 2a. None of the strains of S. flexneri 2a and S. Typhi had mutations in gyrB and parE genes. gyrB gene was not detected in any of strains which exclude the possibility of plasmid mediated quinolone resistance in S. flexneri and S. Typhi in this region. All single PGF type A, subdivided into four subtypes A1 to A4, were found in S. flexneri 2a resistant strains suggesting their genetic relatedness. Similar type of findings was observed in case S. Typhi 2a resistant strains.

**Conclusions:** We have reported the mutation in fluoroquinolone resistant S. flexneri 2a and S. Typhi strains in Bangladesh. Increase isolation of ciprofloxacin resistant strains suggests that S. flexneri and S. Typhi are becoming difficult to treat in Bangladesh.

**Targeted Delivery of Magic Bullets by the Use of Mechanized Nanoparticles**

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**Background:** Bacteria fragilis group are one of the most clinically important group among anaerobic bacteria. Related infections are usually mixed infection with several anaerobic and aerobic bacteria. Most of the isolates in B.fragilis group produce ß-lactamase with a variety spectrum of substrate specificity and it may affect activity of the ß-lactams used. Besides ß-lactam resistant, B. fragilis group shows resistance to wide range of antimicrobial agents. On the other hand, initial chemotherapy for anaerobic infection is necessarily empiric because it takes time to define isolated anaerobes and their susceptibility. Because of the wide range of resistance in B.fragilis group, useful agents for empiric therapy are restricted. Carbapenem(Carb) are useful and important agents for anaerobic infection. We studied on Carb resistant strains in B.fragilis group.

**Methods:** Sensitivity of isolates was determined by agar dilution methods. Production of metallo-ß-lactamase (MBL) in Carb resistant strains was detected by using ß-lactamase activity or sodium menadionic acid (SMAC: Eiken, Tokyo, Japan) disk method. Presence of ß-lactamase was detected by PCR.

**Results:** In ca.1200 strains isolated from 1987 to 1994, we detected 38 resistant strains (B.fragilis 23, non-B.fragilis 15). MBL production was detected in 9 strains of B.fragilis. MICs of imipenem in MBL (+) strains were higher (≥ 64 µg/mL) than that of MBL (-) resistant strains. MBL (+) strains were resistant to other ß-lactams including cefoxitin and ß-lactam inhibitor(BL)ß-lactam, reflecting substrate specificity of MBL. Most of MBL (-) strains (especially in non-B.fragilis) were rather resistant to other ß-lactams including BLß-lactam and cephapirin. Resistant rate to clindamycin in these strains were ca. 80%, although the rate in whole B.fragilis group isolated in the same period was 22%. Among 120 strains isolated after 2000, three MBL (+) B.fragilis and 7 MBL (-) carbapenem less-susceptible strains (MICs of carbapenems; 4–16 µg/mL) were detected.

**Conclusions:** 1) MBL (+) strains showed higher Carb MIC than that of MBL (-) Carb resistant. 2) MBL (+) type may include more often and distribute more widely in B.fragilis group than MBL (-) type. 3) MBL (+) type may be regarded to wide-range of antimicrobial agents. 4) Not only MBL (+) type but also MBL (-) type strains should be monitored.
Xyitol Resistance of Streptococcus mutans Appears To Be An Unexpected Benefit To The Human or Rodent Host

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Background: S. mutans is the prime cariogenic bacterium. Xyitol, a sugar substitute, inhibits its glycolytic metabolism, transmission from mother to child, and induction/evolution of caries. However, xylitol-resistant mutants arise in the mouths of habitual consumers. There are no genotypic data on these mutants and no reports on their cariogenicity (virulence). S. mutans mutants have been constructed that are defective in fructose transport and one of these is also defective in xylitol transport. The mutant of one fructose PTS system that can transport xylitol (fru1) is notably resistant to growth and glycolytic inhibition by xylitol, unlike either its wild type (WT) progenitor, other fructose transport mutants, or reference mutants streptococcal strains.

Methods: We characterized the virulences of WT and engineered fructose transport-defective strains, one of which also fails to transport xylitol, using rats initially free of indigenous mutans streptococci. These rats ate a high sucrose diet 2000 supporting maximal cariogenicity. S. mutans UA159, its isogenic double crossover deletion mutants of fructose PTS (fru1), (fru2), and a double fructose PTS mutant (fru1/fru2), and a sucrose phosphomonoesterase mutant (gfa) were simultaneously studied in TANSPFOM/OMASFBR rats. Some rats were un inoculated and some were inoculated with reference S. mutans strain 104495.

Results: In two separate experiments, all S. mutans strains heavily colonized the rats, however, the recoveries of the fru1 mutant from sonicates of the rats' teeth post mortem were decreased, by comparison with other mutants and its WT. Mutants defective in fru1, fru2, and fru1/fru2 partially lost cariogenicity on experiment. The mutant resistant to fructose PTS (fru1) that fails to transport xylitol, is resistant to growth and glycolysis inhibition by xylitol, and loses some of its ability to colonize the teeth and to cause deep lesions. These results have been reported in extenso by Tanzer et al. J Dent Res 85:369-73,2006. The results suggest that emergence of xyitol resistance among S. mutans strains colonizing habitual xylitol users may, in fact, be of benefit to the host. Supported by grants U54-4/00402 and NIH DE-12236.

The clinical role of the statins in surgical neurosciences

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Background: Statins are structural analogs of hydroxy-methylglutaryl Co A reductase, a repressive enzyme in the cholesterol pathway. They are used for treating patients with the full or mutant especially loss ability to induce decay deep into the teeth. The control gfa mutant did not lose virulence.

Conclusions: Fructose transport via the PEP-dependent fructose PTS of S. mutans UA159 contributes to virulence in sucrose-fed rats, but there are stress specific mutation effects on the ability of this cell to colonize the teeth. An engineered fructose PTS mutant (fru1) that fails to transport xylitol, is resistant to growth and glycolysis inhibition by xylitol, and loses some of its ability to colonize the teeth and to cause deep lesions. These results have been reported in extenso by Tanzer et al. J Dent Res 85:369-73,2006. The results suggest that emergence of xyitol resistance among S. mutans strains colonizing habitual xylitol users may, in fact, be of benefit to the host. Supported by grants U54-4/00402 and NIH DE-12236.

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The potential use of glycoconjugates (carbohydrate-bearing polymers) is receiving considerable attention for biomedical and pharmaceutical applications. Among cellular targets by glycoconjugates, macrophages are ideal, since they play a central role in inflammation and act as reservoirs for microorganisms that are involved with deadly infectious diseases. In this context, we studied effects of glycoconjugates contributing to the inhibition of Bacillus spores and neutralization of toxins during phagocytosis. The effects of glycoconjugates were studied under the following three conditions, namely a) prior to b) during, and c) following spore-macrophage contact with B. anthracis recombinant toxins and spores during phagocytosis. Post-phagocytosis studies involved colony forming units, microscopic observation, macrophage viability, cytotoxicity, and Caspases release assays.

Glycoconjugates promote inhibition of spores and neutralization of toxins by macrophages. Macrophages effectively neutralize spore and/or toxins-induced macrophage cell death, while increasing their activation level. This results in higher phagocytosis rate of spores, toxins neutralization, and macrophage viability. Even after being bound to spores/ toxins on one side, glycoconjugates serve as chemoattracant for macrophages on the other side. Macrophages become more prone to adhere to glycoconjugate-coated spores/toxins, resulting in decreased lactate dehydrogenase and Caspases, in increased nitric oxide production, phagocytosis, and killing of spores and neutralizing toxins.

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Bait bullets controlling Bacillus anthracis: from spore inhibition to toxins neutralization and prevention of spore- and/or toxin-induced cell death

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The potential use of glycoconjugates (carbohydrate-bearing polymers) is receiving considerable attention for biomedical and pharmaceutical applications. Among cellular targets by glycoconjugates, macrophages are ideal, since they play a central role in inflammation and act as reservoirs for microorganisms that are involved with deadly infectious diseases. In this context, we studied effects of glycoconjugates contributing to the inhibition of Bacillus spores and neutralization of toxins during phagocytosis. The effects of glycoconjugates were studied under the following three conditions, namely a) prior to b) during, and c) following spore-macrophage contact with B. anthracis recombinant toxins and spores during phagocytosis. Post-phagocytosis studies involved colony forming units, microscopic observation, macrophage viability, cytotoxicity, and Caspases release assays.

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Acknowledgement: National Science Foundation EPSCoR RII Grant “Arkansas AASET Initiatives” (#EPS-0701890) and the Arkansas Science & Technology Authority; SURF: 2008 American Society for Microbiology UTF and UTC and McNair programs; Kathleen Thomsen Hall Charitable Trust Grants
Micronutrient Interactions in Health and Oxidative Stress Conditions

Structure and Antimalarial Activity of Immunomodulator P-MAPA

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Background: The nutrition research during last two decades has evolved the newer dimensions of protective role of micronutrients in diseases related to oxidative stress and their interactions with each other.

Methods: Linkages of micronutrients have been extensively studied by our group in foods as well as through in vitro systems and in animal and human models.

Results & Conclusions: Riboflavin enhanced the bioavailability and uptake of food zinc and copper and had beneficial effect on iron and zinc utilization in weaning mice. Supplementation of nicotinic acid enhanced bioavailability of iron as observed through in vitro, animal and human studies. Absent absorption of zinc in healthy adults was inhibited by thiamine and ascorbic acid and copper absorption was inhibited by phosphoryl, chromium and nickel. In the human ileostomy model, dietary riboflavin had significant positive association while intake of iron and manganese showed weak negative associations with beta-carotene absorption from vegetable meals. Factors influencing dietary and erythrocyte forms of iron and manganese showed weak negative associations with beta-carotene uptake from plasma levels of selenium, zinc, and copper. Further, in a prospective study on supplementation of greens in healthy adults (40) multiple regression analysis indicated significant association of percent change in plasma zinc with intakes of zinc, riboflavin, iron, ascorbic acid, beta-carotene and copper. In a study on antioxidant potential of commonly consumed fruits and vegetables (96), ascorbic acid levels were associated with inhibition of lipid peroxidation (r = 0.32, p<0.05) and fumos acid chelating activity (r = 0.43, p<0.01). In a cataract study, plasma CATS, a measure of oxidative stress, showed a negative and highly significant correlation with lens opacity (r=0.30, p<0.05). Diabetic cataract patients exhibited inferior plasma levels of selenium, zinc, ceruloplasmin, superoxide dismutase and retinol as compared to cataract patients without diabetes. Further, multiple regression analysis indicated association of intake of iron, beta-carotene, ascorbic acid, polyphenols and inositol hexa-acid in foods as well as through

CYP1A1, GST Gene Polymorphisms and Risk of Chronic Myeloid Leukaemia

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Background: Associations between polymorphisms for genes encoding enzyme involved in biotransformation of xenobiotics and susceptibility to several cancers have been shown in several studies. The aim of the present study is to investigate the influence of cytochromes P450 (CYP450) 1A2,1C2 and Glutathione S-transferases (GSTs) (T1 and M1) gene polymorphisms in susceptibility to chronic myeloid leukemia (CML).

Methods: The frequency of CYP1A1 I105 allele and of GSTT1 and GSTM1 homozygous deletions was examined in 107 patients with CML and 132 healthy controls by PCR and/or PCRRFLP methods using blood samples.

Results: The frequency of CYP1A1 I105 allele was found to be 19.2% in CML patients and 4.4% for controls, indicating that persons carrying this allele had an increased risk of CML (OR = 5.10, 95% CI: 2.60–9.97) (Table 1). The frequency of individuals carrying the GSTT1 null genotype was higher among CML patients (40.2%) compared to controls (19.2%) (OR = 2.82, 95% CI: 1.58–5.05; p<0.001). Therefore, GSTT1 present genotype may be a protective factor for CML. Although GSTM1 null genotype frequency was slightly higher in the patient group (44.9%) than in the controls (42.3%), this difference was not statistically significant (OR = 1.11, 95% CI: 0.66–1.86; p = 0.695). Individuals with GSTM1 null genotypes without the T allele have a 5.981 higher risk for CML than those who have the T allele (Table 2).

Conclusions: This data suggests that polymorphic CYP1A1 and GSTT1 genes appear to affect susceptibility to CML.

Mode of Action of a Naphthoquinone on Tuberculosis

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Background: Euclia natalensis is a shrub or small to medium size tree, which occurs in southern Africa. The roots of it are used by the indigenous people of southern Africa for various bacterial infections. It was showed that six naphthoquinones from this tree have activity against Mycobacterium tuberculosis. One of them, 7-methyljuglone, has very similar structure to menaquinone. In the electron transport process of Mycobacterium species, electrons are transported by menaquinone. Aim: To generate homology model of one of the electron transport system enzymes, cytochrome b from M. tuberculosis, and to investigate the enzyme’s possible interactions with the bioactive anti-tuberculosis compound isolated from plant Euclia natalensis.

Results: Preliminary homology model of M. tuberculosis cytochrome b is calculated by the program MODELLER by using the crystal structure of R. sphaeroides.

Conclusions: Preliminary results revealed that part of the ligand steatinallati located in the cytochrome b of R. sphaeroides crystal structure has a very similar structure to our compound. In laboratory side, it was shown that the minimum inhibitory concentration (MIC) value on the H37Rv M. tuberculosis strain, of the compound is 0.8 g/ml which is comparable to positive drug control rifampicin (0.125 g/ml) and better than ethambutol (1.250 g/ml).

All abstracts are listed in alphabetical order of the presenting author.
And what if we used for therapeutic drug delivery, the “magic bullets” that Dicytostelium discoides cells expel as a multidrug resistance mechanism?

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In 1935, Raper brought the non-pathogenic eukaryotic amoeba Dicytostelium discoidum to the attention of the microbiological scientific community. More than 6 decades later, in 1999, the NIH (National Institutes of Health, USA) decided to add this micro-organism as a new model for biomedical research. Already in 1990, we used Dicytostelium to find out by which mechanism the cells get rid of harm/oxygen, the potent carcinogenic compound of tobacco smoke. We found that, like many human multidrug-resistant cells. Dicytostelium cells do have a multidrug resistance pump, the P-glycoprotein (170 kDa). However, this pump proved to be non-active and therefore not responsible for drug resistance in Dicytostelium cells. Thus, we had to search for a new mechanism mediating drug resistance. We found that Hochet 33432 (HO3432), a DNA-specific matter, was not vitally staining the nuclei, thus giving evidence of Dicytostelium cell nuclear resistance. We observed that when Dicytostelium cells are grown in the presence of HO3432, they expel extraordinarily vesicles loaded with the dye. Such a vesicular pathway of detoxification was indeed the new resistance mechanism we had been looking for.

The next question became: could these biological dye-loaded vesicles be used as a dye-vector to by-pass Dicytostelium nuclear resistance? Experimental data obtained in vivo and also with K562r, a human leukaemia multidrug-resistant cell line, allowed us to propose Dicytostelium vesicles as a promising non-viral drug delivery tool.

As a first approach to therapeutic application, we chose hypericin, a photosensitizer for antitumoral photodynamic therapy. The biological loading of vesicles with hypericin was first controlled. Then, the vesicle-mediated drug transfer was studied, using two human cell lines as target cells, skin fibroblast (HS68) and cervix carcinoma cells (HeLa). Altogether, these data prove that the Dicytostelium extracellular vesicles could be used as "magic bullets" for transferring a therapeutic molecule within human cells.

**Comparison of therapeutic effects of Reboxetine and Methylphenidate in children and adolescents with Attention-Deficit/Hyperactivity Disorder**

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**Background:** Disregulation of biogenic amines especially of norepinephrine and dopamine has been proposed in pathophysiology of attention-deficit/hyperactivity disorder (ADHD). According to primary and secondary metabolisms in a membrane-surface liquid culture bioreactor called a nano-particulate membrane bioreactor (NMB) to sparged liquid cultures. 2) To assay the influence of membrane thickness on metabolic activities. 3) To scale the NMB up to a size suitable for commercial applications.

**Methods:** A variety of experiments were conducted to measure antibiotic biosynthesis, nutrient consumption and biomass growth in various NMB rigs, and were compared directly to sparged submerged cultures fed identical nutritional broth and inoculated at the same temperature. The effect of varying the thickness of the membranes in the NMB was also assayed with respect to primary and secondary metabolisms in the NMB with the yields increasing through the experiment. After the first batch there was no lag before biosynthesis resumed, so primary and secondary metabolisms were concurrent. Scale-up has reached 1,150 m³, capable of treating 340 L/day depending on the application. The NMB can be repeatedly steam sterilized and can be made of materials compatible with pharmaceutical manufacture.

**Conclusions:** 1) The concurrent solid and liquid phases in the NMB enables obligate aerobic microbes to grow in direct contact with air, while communicating with a liquid phase for easy control of pH, temperature and nutrient and precursor concentrations, easy separation of products downstream, efficient biomass retention and greater yields and production rates, which makes the NMB an ideal technology for biosynthesis of primary and secondary metabolisms. 2) Productivity in the NMB increases with thinner membranes. 3) The NMB has been scaled up to be suitable for a wide variety of commercial applications.
Isatin (2,3-dioxo-indole) and its Analogues as New Antipyretic Agents

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Background: Most of the antipyretic drugs available are acting mainly on blocking the arachidonic cascade forming PGE2. From our previous experience isatin (2,3-dioxo-indole) is able to prevent hyperthermia by blocking the action of PGE2 also. Methods: In the present work the action of isatin and isatin analogues (5-methylisatin, 6-hydroxyisatin, 7-ethylisatin, N-acetylisatin) have been tested on prostaglandin E2 (PGE2)-induced hyperthermia in mice and rats. Two forms of induced hyperthermia have been tested. When the PGE2 was given simultaneously with the isatin or analogues, the development of hyperthermia was tested, when the test compounds were given 30 min following PGE2 administration, the action on already existing hyperthermia was measured in mice and in rats. The temperature of the animals was measured in the colon.

Results: The results demonstrate that isatin in a dose of 12.5 mg/kg ip is able to block the initiation and in a dose of 25.0 mg/kg ip attenuate the PGE2-induced existing hyperthermia in rats. In mice 3.12 mg/kg ip isatin can block the development of PGE2-induced hyperthermia, while in a dose of 12.5 mg/kg ip can attenuate the existing hyperthermia. 5-Methylisatin in a dose of 3.36 mg/kg ip can block the development of hyperthermia and in the dose of 13.44 mg/kg ip can attenuate the existing PGE2-induced hyperthermia in rats. In a dose of 0.21 mg/kg ip can block the initiation of hyperthermia and in a dose of 6.72 mg/kg ip attenuates the existing hyperthermia in mice.6-Hydroxy-isatin in a dose of 0.40 mg/kg ip blocks the development and also the existing hyperthermia in rats. In mice 5.2 mg/kg is able to block the development of hyperthermia and in a dose of 10.4 mg/kg ip attenuates the existing hyperthermia. 7-Ethylisatin in the dose of 0.112 mg/kg ip blocks the initiation and also the existing hyperthermia in rats. In mice both the initiation and also the existing hyperthermia can be blocked by the dose of 0.088 mg/kg N-Acetylisatin dose of 0.036 mg/kg ip blocks the initiation and the dose of 0.384 blocks the existing hyperthermia in rats. 0.005 mg/kg ip blocks the initiation of hyperthermia, while the dose of 1.024 mg/kg blocks the existing hyperthermia in mice.

Conclusion. The results demonstrate that not only isatin but the isatin analogues can also block the PGE2-induced hyperthermia and that 7-ethyl- and N-acetylisatin are the most effective compounds in both blocking the development of hyperthermia and also in attenuating the PGE2-induced hyperthermia.

Application of Population Pharmacokinetic Analysis and Monte Carlo Simulations Method in Drug Phenotyping. Assessment of Cytochrome P450 1A2 Activity in a Population of Adult Non-Related Caucasians from Sparse Data

TERZIVANOV DN, BOZHINOVA KV

Background: Already published data of the authors have unambiguously demonstrated the ability of the Nonparametric Expectation Maximisation (NPMEM) method of population pharmacokinetic modelling to deal with sparse data in estimating systemic caffeine clearance (CLS1) for monitoring and evaluation of cytochrome P450 (CYP) 1A2 activity in a population of adult non-related Caucasians. The results of this investigation have shown that NPMEM analysis is a reliable and sufficiently sensitive method for clinical testing of liver function even in presence of renal dysfunction, using CYP1A2 as a biomarker. On the other hand, these results have revealed the abilities of the NPMEM method as suitable and relevant for large-scale epidemiological studies with regard to phenotyping cancer or other high risk populations by monitoring their CYP1A2 activity based on random sparse data. The method can deal even with mixed populations (smokers and non-smokers) in discriminating multicomponent distributions with departure from normality, which the parametric methods have failed to do.

Methods: Large-scale epidemiological study was simulated using the method of Monte Carlo simulations (MCS) in order to explore the applicability of nonparametric population pharmacokinetic modeling in phenotyping of CYP1A2 activity by using of CLS1 as a biomarker. Simulated populations from 500 to 1000 subjects were studied. 250 simulated subjects were randomly sampled according to a 10 points sampling scheme and their simulated "measured" plasma caffeine concentrations were further submitted to nonparametric population pharmacokinetic analysis.

Results: The resulted distribution of CLS1 was clearly trimodal. Each of the clusters ("High", "Intermediate", and "Low") had normal distribution of CYP1A2 activity. Obviously, in no clinical setting it would be possible to sample randomly 250 subjects.

Conclusions: These results reveal the exceptional usefulness of MCS in drug phenotyping when dealing with clinical setting data which are, by their very nature, limited and sparsely distributed.

Efficacy of 1% topical cyclosporine in the treatment of severe vernal keratoconjunctivitis in childhood

APPLICATION OF POPULATION PHARMACOKINETIC ANALYSIS AND MONTE CARLO SIMULATIONS METHOD IN DRUG PHENOTYPING. ASSESSMENT OF CYTOCHROME P450 1A2 ACTIVITY IN A POPULATION OF ADULT NON-RELATED CAUCASIANS FROM SPARSE DATA

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Introduction: Corticosteroids and high-concentrated cyclosporine eyedrops have been used for treatment of severe vernal keratoconjunctivitis (VKC) cases. The purpose of our study was to verify the efficacy of 1% topical cyclosporine in improving severe form of VKC in childhood and investigate for factors affecting the response to therapy.

Material and Methods: We conducted an open trial involving 197 children with severe VKC, who received topical cyclosporine 1% for 4 months. Ocular subjective symptoms and objective signs were scored in all children at entry, 2 weeks and 4 months. Skin prick tests and microscope endothelial cells evaluation were also performed; serum IgE and cyclosporine levels were assessed.

Results: The mean score values for severity of subjective symptoms and objective signs were significantly decreased after 2 weeks, and 4 months, compared with those at entry (p<0.001) in all children. Cyclosporine serum levels were not detectable at the end of therapy, nor were endothelial corneal cells damaged. Patients who started the therapy at the beginning of the disease and/or received long-term regimen of treatment with cyclosporine had a faster improvement of ocular signs and symptoms, compared to all other patients.

Conclusion: Our findings suggest that 1% cyclosporine concentration administered topically at the beginning of the disease and for a long-term period might be the most effective treatment to control symptoms and local inflammation in severe forms of VKC in childhood.
Nephrotoxicity of immunosuppressive drugs, new insight

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Background: Organ transplantation is the best treatment, if not the only, of end stage organ failure. However, some assumptions within the incidence of acute rejection. The FACS (cyclophilin A) is a new marker for reclassification of donor-recipient status. Cyclosporine A is the most commonly used immunosuppressive drug. New insights in nephrotoxicity mechanisms also have been demonstrated. For example, in vitro data demonstrated that F-glycosylation may play a critical role in protecting renal epithelial cells from cyclosporine A (CSA) toxicity. The molecular mechanisms, transcriptional profiles of human proximal tubular cells exposed to CSA found that it preferentially alters biological processes located at the cell membrane, such as ion transport or signal transduction. No significant changes in gene expression were found in cells treated with CSA. In conclusion, CSA induces cell death; however, the mechanisms involved in this process are not yet completely understood. The term paradigm shift, as introduced by Thomas Kuhn, describes a change in a well-developed area under a new theory of science. A change in a certain thought-pattern is usually the result of a long process. Applying the paradigm shift model to the measurement of concentrations of anti-infective drugs at the site of infection, we indeed observed a remarkably slow shift in thinking and acting even in the face of compelling reasons for change. For several decades anti-infective drug concentrations at the site of infection have been primarily measured by taking a whole tissue biopsy, grinding it, determining the total concentration in the homogenate, comparing it with the corresponding blood sample, and then judging a drug's clinical value from such a measurement. As has been shown, homogenizing biopsy samples results in a mixture of different pharmacological compartments, of bound and unbound drug, and, thus, fails to give meaningful information about concentrations at the infection site. As a result, there is a definite need for an improved whooping cough vaccine. Therefore, there is a definite need for an improved whooping cough vaccine. The most likely candidates to fulfill all characteristics are oral or intranasal vaccines consisting of inactivated whole B. pertussis cells, given the fact that an oral vaccine has already shown proof of protection in a phase III study without adverse events, and that an intra-nasal vaccine has shown proof of concept in a phase I study. Live attenuated vaccines fulfill many of the 5 characteristics as well, but will most likely take longer to reach the market. At this point it is clear that B. parapertussis component should also be included in an improved whooping cough vaccine, and what the cost-benefit ratio would be.

Whooping cough vaccines: a public health and producer's perspective

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Background: Despite the high vaccination coverage in most of the Western world, the incidence of whooping cough has been increasing in all age groups for the last 2 decades. The global rise in whooping cough cases did not lessen after the introduction of acellular vaccines a decade ago, or the introduction of booster vaccinations in toddlers. Currently registered vaccines are not well equipped to overcome the rising incidence of whooping cough for a number of reasons. Therefore, there is a definite need for an improved whooping cough vaccine. Methods: This paper selected the following characteristics of an improved vaccine. An improved whooping cough vaccine should (1) enable all age groups to be vaccinated with B. pertussis circulating strain antigens, (2) protect against whooping cough induced by B. parapertussis, (3) enable infants to be vaccinated earlier, (4) cause minimal adverse events after repeated vaccination, and (5) protect longer than currently registered vaccines. A number of scenario's and corresponding vaccine compositions that comply with (part of) these characteristics were examined, using time to market, costs and risks as constraints. Results & conclusions: The most likely candidates to fulfill all characteristics are oral or intranasal vaccines consisting of inactivated whole B. pertussis cells, given the fact that an oral vaccine has already shown proof of protection in a phase III study without adverse events, and that an intra-nasal vaccine has shown proof of concept in a phase I study. Live attenuated vaccines fulfill many of the 5 characteristics as well, but will most likely take longer to reach the market. At this point it is clear that B. parapertussis component should also be included in an improved whooping cough vaccine, and what the cost-benefit ratio would be.

Evaluating our Performance: Tissue Penetration Paradigm Shift

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The term paradigm shift, as introduced by Thomas Kuhn, describes a change in a well-developed area under a new theory of science. Such a change in a certain thought-pattern is usually the result of a long process. Applying the paradigm shift model to the measurement of concentrations of anti-infective drugs at the site of infection, we indeed observed a remarkably slow shift in thinking and acting even in the face of compelling reasons for change. For several decades anti-infective drug concentrations at the site of infection have been primarily measured by taking a whole tissue biopsy, grinding it, determining the total concentration in the homogenate, comparing it with the corresponding blood sample, and then judging a drug's clinical value from such a measurement. As has been shown, homogenizing biopsy samples results in a mixture of different pharmacological compartments, of bound and unbound drug, and, thus, fails to give meaningful information about concentrations at the infection site. As a result, there is a definite need for an improved whooping cough vaccine. Therefore, there is a definite need for an improved whooping cough vaccine. The most likely candidates to fulfill all characteristics are oral or intranasal vaccines consisting of inactivated whole B. pertussis cells, given the fact that an oral vaccine has already shown proof of protection in a phase III study without adverse events, and that an intra-nasal vaccine has shown proof of concept in a phase I study. Live attenuated vaccines fulfill many of the 5 characteristics as well, but will most likely take longer to reach the market. At this point it is clear that B. parapertussis component should also be included in an improved whooping cough vaccine, and what the cost-benefit ratio would be.

EHRILICH II – 2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich
Niemberg, October 3-5, 2008

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Antifungals in the Treatment of Candidiasis Eruption in Oral Autoimmune Diseases
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Background: Candida infection has been reported as the common lesion eruption during treatment of oral autoimmune diseases such as oral lichen planus (OLP), lupus erythematosus (LE), pemphigus vulgaris (PV) and mucous membrane pemphigoid (MMP) with topical and systemic steroids. Aims: 1) To study the incidence of candidiasis eruption after treatment with steroids. 2) To investigate the effectiveness of topical antifungals. 3) To know the side-effects of topical antifungals.

Methods: A retrospective study included 297 patients (female=236; male=61), age ranged between 18-80 years (Mean 55.69). Free consent was obtained. The samples were collected from the oral cavity and confirmed by histopathological or immunochemical tests. Topical antifungals were used in 40 and 25 cases.

Results: We have identified candidium-(III)-based contrast agents that exhibit a 3-5 fold signal enhancement after being cleaved by MFECP1, a recombinant antibody-enzyme fusion protein of an anti-carcinoembryogenic antigen single-chain Fv antibody and the bacterial enzyme carbonylasepide B2. The structure of this enzyme has been optimized to improve the compound's stability and rate of CPG2 hydrolysis.

Conclusions: New enzyme-activated MRI contrast agents have been produced that once evaluated for use in humans could be utilised to optimise ADEPT or GDEPT treatments on a patient by patient basis.

Innovative Method For Quality Control of High Molecular Weight Semi-synthetic Vaccines
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Background: Robbins, Schneerson et al. developed conjugated meningitis vaccines (Hb) for infants [1]. Earlier preparations varied in immunogenicity. For accelerated testing of vaccines, analytical methods based on physical parameters were developed from 1983-95. These techniques [2] remain a promising tool for vaccine quality control and for predicting vaccine effectiveness (recently reviewed in [3]).

Methods: Horizontal 2-0 subdermic-type agarose electrophoresis was employed, a method developed by Siever [4] for the separation of intact and capsular lipopolysaccharides. The method was then used for data analysis and image processing [2].

Results: Original images (left) show characteristic patterns for vaccine preparations (I-III) and two carbosilipolysaccharide samples (S) used for standardization. These images were transformed (right) to a coordinate system of particle radius vs. free mobility (surface net charge). No distinct zone patterns can be seen (I-III), since sizes of vaccine particles vary continuously over a wide range (polysilpores) due to randomizing processes in the vaccine preparation (crosslinking and sonication).

Conclusion: 1) The vaccine patterns are a fingerprint of the preceding vaccine preparation process and, therefore, can be used for purposes of quality control. 2) Results are available within 1-2 days, whereas immunological testing may take several weeks. 3) Samples II and III with particle sizes larger than 30 nm radius were effective vaccines. Sample II contains a mixture of vaccine batches. 4) The technique cannot only be used for Hib conjugated meningitis vaccines, but also for other high molecular weight vaccines.

References:
The in vitro effects of Thymoquinone on human endometrial adenocarcinoma cells

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Background: In this study, it was investigated whether Thymoquinone (TG) has the effect on women endometrial adenocarcinoma cells as in vitro.

Methods: It was used frozen vial human endometrial adenocarcinoma (KLE) cells that obtained from American Type Culture Collection (ATCC) in this study. KLE cells were treated with TG for various dosages.

Results: According to experiments, it was founded that TG has toxic effect in all dilutions until that 300 micro molar (μM). Especially, TG had blocking effect on growing in number of KLE cells.

Conclusions: It was determined that TG that composition of Nigella sativa has blocking effect on growing in number of endometrial adenocarcinoma cells as dose depending in cytotoxicity experiments that in vitro.

Dissecting the DNA Base Excision Repair Pathway: Implications for Cancer Therapy

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Background: The base excision repair (BER) pathway removes DNA bases damaged by oxidizing and alkylating agents, as well as mispaired bases. The first step of BER is initiated by DNA glycosylases that recognize and excise aberrant bases. Subsequently, the resulting abasic site is processed by other specialized BER enzymes that generate a single nucleotide gap and replace the missing nucleotide.

The BER pathway is clinically important as it prevents deleterious mutations and also processes DNA lesions produced by anticancer drugs. Thymine DNA glycosylase (TDG) is a BER enzyme dedicated to the repair of damaged cytosine-guanine (CpG) dinucleotides. TDG and other BER enzymes have been implicated in epigenetic control of gene expression by gene-specific CpG demethylation. TDG also regulates gene expression by direct interaction with the transcription machinery. Interestingly, this versatile enzyme also modulates removal of 5-fluorouracil from DNA and may determine the sensitivity of cells to this widely used anticancer drug. TDG is posttransationally modified by sumoylation, phosphorylation and acetylation. In exploring the potential of TDG as a drug target, we have sought to decipher how these modifications regulate various TDG functions.

Methods: We have employed cell imaging techniques, cell-based reporter gene assays and biochemical analysis to investigate the regulatory roles of TDG posttranslational modifications.

Results: We demonstrate that posttranslational modification of TDG dramatically affects TDG subnuclear localization as well as the enzyme activities for anabolism and catabolism, thereby altering the transcriptional and DNA repair functions of this enzyme. Importantly, we have identified specific kinase signaling pathways responsible for phosphorylation of TDG in living cells and show that phosphorylation acts antagonistically to acetylation to regulate DNA damage processing. These findings have allowed us to design mutator versions of TDG that display more potent DNA repair functions.

Conclusions: These findings suggest that multiple post-translational modifications and therefore different signaling pathways regulate the biochemical properties and subcellular localization of TDG. The interplay of these covalent modifications allow for exquisite regulation of a DNA repair pathway integral for genome stability.

The Acute Effects of Statins in an Experimental Model of Renal Ischemia-Reperfusion Injury

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Methods: Acute renal failure. We aimed to compare the acute effects of different statins in an experimental model of renal ischemia-reperfusion injury (45 min of I + 4 h of R), were pretreated with simvastatin or pravastatin (1 mg/kg, i.v.), 30 min before I, 30 min before R or 5 min after reperfusion. Blood and kidney tissue samples were taken at the end of experiment and selected parameters of glomerular and tubular function were assessed. Statistical analysis was carried out.

Results: Simvastatin-treated rats had significantly reduced serum creatinine concentrations, fractional excretion of sodium and total histological score in comparison with controls (up to 80%, 80% and 40%, respectively). The acute protective effects of simvastatin (1 mg/kg) did not depend on the time of injection and the dosages used. On the other hand, pravastatin (1 mg/kg) significantly more effective than simvastatin in reducing IR-induced changes of glomerular and tubular function, especially regarding total histological score.

Conclusions: The acute pretreatment with a single dose of statin may ameliorate renal impairment and allow earlier recovery from IR injury. However, it seems that statins are not equally effective in reducing such an injury.

Clinical Role of Fluorouracil Metabolizing Enzymes and Optimal Duration in Chemotherapy with Tegafur

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Objectives: Response rates of 5-fluorouracil (5FU) and tegafur likely differ individually due to differences in the enzyme activities for anabolism and catabolism. Orotate phosphatase transferase (OPRT) is an essential enzyme for activation of 5FU. Dihydropyrimidine dehydrogenase (DPD) is a rate-limiting enzyme for degradation of 5FU. To obtain a high percentage of response without side-effects, it would be important to evaluate the enzyme activities and to estimate the optimal duration of chemotherapy.

Patients and Methods: The study included 160 patients, whose colorectal cancer stage II to IV was treated with uracil and tegafur (UFT) after surgery. (1) OPRT and DPD expressions were evaluated using immunohistochemistry. Relationships between their expressions and clinicopathological features. Survival curves were calculated using Kaplan-Meier method. (2) Using the collagen gel droplet embedded drug sensitivity test (CD-DST), we estimated the optimal duration of chemotherapy. Area under curve (AUC) was calculated with the inhibition rates assessed in variety of time and concentration in the tests. The AUC value for 50% inhibition (AUC50) was also calculated. The AUC50 of 5FU infusion (250 mg/m2/day), UFT (400 mg/m2/day) were about 1.7 and 1.4 (micro-gram x hr/mL). Based on these values, the duration to attain the AUC50 for 5FU and UFT were estimated.

Results: (1) OPRT expression showed a negative correlation with advances in cancer stage, though DPD expression showed positive correlations. The patients survival were better in those OPRT+ than those OPRT− (p<0.004). In regard to the combination of these expression, the best survival curve was obtained for the OPRT+ DPD− group and the worst was OPRT− DPD+. The AUC50 in 38% of the patients ranged < 100; 43% ranged 100-1000; 13% ranged 1000-10000; and 6% ranged > 100000 respectively. Therefore, the duration to attain the AUC50 by 5-FU infusion ranged < 10 weeks in 37% patients; 10-100 weeks in 44 %; and longer than 100 weeks in 19% respectively. The durations to attain the AUC50 by UFT ranged < 6 months in 55% patients; 6-12 months in 13 %, and longer than 12 months in 32% respectively.

Conclusions: OPRT expression related with better prognosis, although DPD expression were related with poor prognosis. The use of several determinants of response may identify a high percentage of responding patients. The optimal duration of chemotherapy with 5FU or tegafur may be estimated using CD-DST, though it differs in patients.
Developing a magic bullet against P-glycoprotein-mediated drug resistance
by exploiting mechanism

TOMBLINE G1,2, HOLT J1,2, GANNON MK1,2, SAWADA G1,2, RAUB TJ1,2,
DONNELLY D1,2, WETZEL BJ1,2, YI M1,2, NYGREN CL1,2, DETTY MR1,2

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Background: Multidrug resistance (MDR) is a major obstacle for the treatment of
cancer as well as bacterial infections. Efflux pumps are often the root cause of
MDR. The prototypical human multidrug resistance efflux pump P-glycoprotein
(Pgp) couples drug export to ATP binding and hydrolysis. Details regarding drug
trajectory, the molecular basis for coupling, and factors governing transport
rate/efficiency remain unknown. Our goal is to reveal these details. Rhodamine
dyes have been used as drug mimics to assay Pgp-mediated transport. Herein, we employ novel synthetic analogues of Tetramethylrhodamine (TMR) to
probe Pgp mechanism. A unique advantage is our ability to tease apart the
capacity of a drug to confer coupling to ATP catalytic sites versus transport
rate/efficiency.

Methods: A library of TMR analogues was constructed and their effect on purified
lipid-activated mouse MDR3 Pgp ATPase was determined. Coupling to ATP
catalytic sites was also determined by their ability to promote ADP/PI trapping.
ATP occlusion in "catalytic carbonyl" mutant Pgp, as well as through inhibition of
verapamil-dependent ATPase. Effect on drug transport was measured in vivo
with Madin-Darby Canine Kidney monolayer cells that express Pgp (MDCK-
MDR1).

Results: Nearly three orders of magnitude variation in ATPase stimulation within
the library was apparent. Importantly, the concentration of TMR required for
coupling to the ATP site does not clearly correlate with turnover rate. In some
cases, the ability to promote ATP occlusion is associated with robust turnover.
Several other analogues stimulate ATP occlusion relatively well but displayed very
slow turnover rates. This class also appeared relatively effective as Pgp
inhibitors/modulators in vitro and in vivo.

Conclusions: Drug recognition may not be apparent from ATPase stimulation
which may manifest as equivalent to (or below) basal values. Promotion of ATP
occlusion appears to reveal the true affinity of a drug for Pgp and this does not
 correlate with transport rate. Molecules that promote ATP occlusion effectively yet
confer slow turnover rates are good leads for development of competitive
inhibitors.

Aminoglycoside Derivatives as Drug Transporters: Delivery Magic Bullets?
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Background: A major obstacle for the development of new therapeutic agents is
drug delivery. Many drug candidates never make it to the clinic because of
inappropriate pharmacokinetic characteristics and sluggish uptake. Delivery
procedures based on passive diffusion across cell membranes encounter problems
due to charged groups, and carriers that exploit endogenous membrane
transporters limit the size of potential drug candidates. Low MW delivery vehicles
due to charged groups, and carriers that exploit endogenous membrane
transporters limit the size of potential drug candidates. Low MW delivery vehicles
that facilitate the uptake of diverse molecular cargos are highly desired.
Guainidinoglycosides, a family of synthetic derivatives where all the ammonium
groups have been converted into guanidinium groups, efficiently
facilitate uptake of cargo into mammalian cells. These carriers can be used as a screening
carrier to test the ability of guanidinoglycosides to deliver large proteins into cells and release them in active form, saporin conjugated to streptavidinylated-
fluorescent conjugates of biotinylated guanidino-neomycin with streptavidinylated-
phycocerythrin-cytochrome were incubated with wild type and mutant CHO cells
defective in glycosaminoglycan assembly. To examine the ability of guanidino-
neomycin to deliver large proteins into cells and release them in active form, saporin
conjugated to guanidino-neomycin-biotin and cell killing was evaluated. This
conjugated toxin was also used to examine the susceptibility of tumor cell lines to
these carriers. Enzyme replacement therapy was explored in MPS fibroblasts with
guanidinoneomycin conjugated to -glucuronidase.

Results: (1) the cellular binding and uptake of guanidinoglycosides at low
concentration depends exclusively on hepatic sulfate (in contrast, the uptake of
arginine-rich peptides follows multiple pathways); (2) guanidinoglycosides are
capable of cellular delivery of high MW and bioactive cargo such as enzymes and
groups of aminoglycosides have been converted into guanidinium groups, efficiently
facilitate uptake of diverse molecular cargos are highly desired. Guainidinoglycosides, a family of synthetic derivatives where all the ammonium
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Results: (1) the cellular binding and uptake of guanidinoglycosides at low
concentration depends exclusively on hepatic sulfate (in contrast, the uptake of
arginine-rich peptides follows multiple pathways); (2) guanidinoglycosides are
capable of cellular delivery of high MW and bioactive cargo such as enzymes and
protein toxins; (3) effective guanidinoglycoside-mediated delivery can be achieved
at low transporter concentrations, with little or no cellular toxicity; and, preliminarily,
(4) certain tumor cell lines (particularly breast cancer) show high susceptibility to
guanidinoglycoside-linked toxins.

Conclusions: Guanidinoglycosides are promising low MW cellular transporters with
high selectivity towards heparan sulfate. These carriers can be used as a screening
tool to explore the surface glycosociology of tumor cell lines as well as for the targeted
delivery of bioactive molecules (e.g., enzymes).
Infant mortality in Hungary was higher than in other European countries; however, the reported incidence of Sudden Infant Death Syndrome (SIDS) has been lower than those for Western Europe and the United States. Childhood immunisation has been reported to be a protective factor for SIDS. In Britain, the change to an earlier immunisation schedule for diphtheria, pertussis, and tetanus appeared to be associated with a shift in the age distribution of SIDS. In 1999, immunisation for Haemophilus influenzae type b (Hib) was introduced for Hungarian infants at the age of 2 months. Data for total infant mortality and SIDS in Hungary was analysed between 1990-2002. Infection was the major cause of death among Hungarian infants followed by SIDS. Following introduction of Hib immunisation, there was a decrease in deaths due to meningitis from an average of 3.5% of all infant deaths between 1990-1998 to an average of 1% of all infant deaths between 1999-2002 (p<0.00). There was also a significant decrease in the proportion of SIDS in the age range >2 months from 48% in the earlier period to 39% after introduction of the vaccine (p=0.03). The decrease in SIDS might be due to decrease in unrecognised Hib infections or to the induction of antibodies by the tetanus toxoid to which the Hib polysaccharide is conjugated that are cross reactive with bacterial toxins implicated in SIDS.

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Liquid chromatography-tandem mass spectrometry assay for determination of raloxifene and its two glucuronide metabolites in human plasma and serum

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Background: Anti-oestrogenic agent raloxifene has recently attracted a lot of attention due to the newly discovered potent breast cancer preventing effects. Unconjugated raloxifene plasma concentrations are very low, \( C_{\text{max}} \) is only 1.36 ng/mL, while the majority of circulating raloxifene is in conjugated form with glucuronic acid. In addition, the raloxifene glucuronide standards were not commercially available. Therefore, the aim of our research was to develop a method for quantification of raloxifene (Ral) and its two glucuronides, raloxifene-6-glucuronide (M1) and raloxifene-4'-glucuronide (M2), in human plasma and serum. Methods: Glucuronides (M1 and M2) were synthesized enzymatically with recombinant human UGT1A1, purified, characterized and used as authentic standards. The assay involves a solid phase extraction (SPE) procedure of 0.5 mL human plasma or serum spiked with haloperidol as internal standard on Strata X cartridges. After elution with 70% acetonitrile and 30% methanol containing 2% formic acid, the samples were dried, reconstituted with 170 µL of initial mobile phase and injected on a Luna C18(2) 50x2.0 mm column. The mobile phase consisted of 0.1% formic acid in acetonitrile (A) and in water (B) with a gradient from 10% to 100% A in 7 minutes. The flow was diverted to waste except from 3.9 to 6.3 min, when it entered the (+)ESI-QqQ. The following mass transitions were from 10% to 100% A in 7 minutes. The flow was diverted to waste except from 3.9 to 6.3 min, when it entered the (+)ESI-QqQ. The following mass transitions were achieved limits of detection were 8, 11 and 6 ng/L for M1, M2 and Ral, respectively. The developed assay proved to be sensitive, accurate and precise. We expect this method can be applied with little modifications also to newer drugs from the SERM family, like bazedoxifene, arzoxifene and lasofoxifene.

Authors’ disclosure statement: The authors acknowledge financial support from the state budget through the Slovenian Research Agency (project P2–0126) Pharmacogenetic study of raloxifene metabolism and transport.

HIV Protease Inhibitor: An Antifungal Agent?

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Background: Protease inhibitors were shown to inhibit Candida albicans adherence to epithelial cells but not endothelial cells. Whether protease inhibitors have any effect on C. albicans adherence to acrylic surface and can be used as an antifungal is still unknown. Aims: The current study aimed to investigate whether protease inhibitors attenuate C. albicans adherence to acrylic surface. The effect of three protease inhibitors, namely Saquinavir, Ritonavir and Indinavir on adherence were compared.

Methods: C. albicans suspensions were pre-treated with different concentrations (0.8, 4, 20, 100 and 500 µM) of Saquinavir, Ritonavir or Indinavir for one hour. The yeast cells were then allowed to adhere to acrylic strips treated with human pooled saliva for another hour (Group A). Adherence was determined by calculating the percentage of cell area over the acrylic surface using an image analyser. Another group with C. albicans not pre-treated with protease inhibitors (Group B) and a control group with no protease inhibitors added (Group C) were also included.

Results: All three protease inhibitors significantly attenuated adherence of C. albicans to acrylic surface. Group B showed significant reduction in adherence compared with Group C. 50% reduction in adherence occurred at concentrations of 100 µM, 100 µM and 20 µM for Saquinavir, Ritonavir and Indinavir, respectively. A dose dependent inhibition of adhesion were observed for all the protease inhibitors in Group A, which was significantly higher in Indinavir than in Saquinavir and Ritonavir. However, such difference disappeared at concentration of 500 µM.

Conclusions: Protease inhibitor had a direct effect on C. albicans pathogenicity; it attenuated C. albicans adherence to acrylic surfaces in a dose related fashion. Moreover, different protease inhibitors exhibited different degrees of inhibition.
Expression of Topoisomerase I and II Protein in Primary Colorectal Cancer; is That the Culprit of Recurrent Disease Following 5-Fluorouracil-based Adjuvant Chemotherapy?

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Background: Human DNA topoisomerases I and II (topo-I and topo-II) are essential for vital cellular processes such as DNA replication, transcription, translation, recombination and repair. Following a chain of observations and pilot studies, we present the findings of a pioneer study, in which topo-I and topo-II expression was correlated with outcome after chemotherapy in primary and relapsed colorectal cancer.

Methods: Patients with colorectal cancer that had recurred, following surgery and adjuvant chemotherapy and underwent a second operation were included in the study. All had undergone surgical resection of the primary tumor and received post-operatively 5-FU-based (5FU-Leucovorin, Mayo Clinic regimen) adjuvant chemotherapy. Tissue was collected at the initial operation from the primary tumor and at the time of recurrence (during the second operation following chemotherapy). All tissue samples were analyzed for levels of expression of both topo-I and topo-II using standard three-step immunohistochemistry on paraffin sections.

Results: Forty patients were included in the study. Levels of expression of topo-I and topo-II were higher in malignant tumors from cancer recurrence compared to primary tumors (p=0.001 for both). There was a statistically significant positive relationship between patients’ age and levels of topo-I (p=0.011) and topo-II (p=0.011) expression.

Conclusions: The study results underscore the role of topoisomerase expression in colorectal cancer and suggest a potential role in tumor recurrence. This model could be further studied, to include other forms of neoplasia and infection, in an effort to elucidate the development of chemotherapy drug resistance, thus optimizing treatment strategies and improving cancer patient care.

Myostatin Inhibiting Peptide Works as a Magic Bullet to Increase Skeletal Muscle Mass and to Ameliorate Muscle Pathology in Muscular Diseases by Transgenic Expression

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Background: Increase of skeletal muscle mass and prevention of muscle atrophy have tremendous medical needs. Myostatin inhibition is capable of increasing skeletal muscle mass and preventing muscle atrophy. It is a promising therapeutic strategy for muscle wasting diseases such as muscular dystrophy and aging. It is also possible that myostatin inhibition will become a novel doping strategy that is difficult to detect in urine or blood tests.

Methods: Myostatin inhibitors include myostatin propeptide, follistatin, and myostatin antibodies. We have developed the myostatin-specific inhibitors derived from follistatin by domain deletion and shuffling. Transgenic mice expressing the myostatin inhibitor, called FSI-I, under the control of a skeletal muscle-specific promoter showed increased skeletal muscle mass and strength (Faseb J. 2008). We crossed FSI-I Tg with mice, a model for Duchenne muscular dystrophy.

Results: Microarray analysis was performed. The skeletal muscles in the mdx/FSI-I mice showed enlargement and reduced cell infiltration. The muscle strength was recovered in the mdx/FSI-I mice. Our data indicate that myostatin inhibition by FSI-I has a therapeutic potential for muscular dystrophy. Microarray analysis showed the remarkable changes of expression of enzymes for fatty acid metabolism such as acetyl-CoA carboxylase (ACC) and carnitine palmitoyltransferase (CPT-1) in skeletal muscle in FSI-I mice. Expression of molecular markers for mitochondria such as cytochrome c and uncoupling proteins increased.

Conclusions: 1) Myostatin inhibitors derived from follistatin are possible candidates of “Magic Bullets” that will increase skeletal muscle mass and may change the nature of our body composition and quality of life. 2) Microarray analysis suggests the changes of expression of metabolic enzymes. 3) Mitochondria numbers in muscles and adipose tissues increase by myostatin inhibition by follistatin-derived molecules.
Severing the Gordian Knot: Are Glycolipids the solution for Effective Vaccines against Malaria and HIV?

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The eradication of global pathogens responsible for endemic and pandemic diseases hinges upon the development of effective vaccines. This is certainly the case for HIV and malaria. However, our inability to elicit “strong and long-lasting” protective T cell responses, particularly CD8+ T cell responses, has been a major obstacle to successful vaccine development. Accordingly, adventitious technologies will likely be critical not only to overcome pre-existing immunity to viral vaccine vectors but also to further enhance vaccine immunogenicity. In our previous studies, a glycolipid, called alpha-galactoceramide (alpha-GaCer), which binds to CD1d molecules and stimulates natural killer T (NKT) cells, was found to display an adjuvant activity and enhance protective CD8+ T cell responses elicited by HIV and malaria vaccines. In search of a more potent adjuvant, we have evaluated a compound library of approximately 100 new glycolipids and have identified several alpha-GaCer analogues that act as powerful NKT cell ligands. The evaluation process includes the testing of ability for glycolipids to stimulate human invariant NKT cells in vitro, as well as their ability to stimulate homologous dendritic cells (DCs). The selected compounds were then inoculated into mice in order to determine their ability to stimulate invariant NKT cells and DCs in vivo. By a series of assays, a few compounds were selected based on their potent biological activities. Finally, a highly novel compound that displays a superlative adjuvant effect against HIV and malaria vaccine platforms in a mouse model. Since CD1d molecules and NKT cells are very much conserved between mice and humans, we anticipate that the lead compound that we identified will improve the efficacy of various vaccines in humans in the near future.

Protective effects of minocycline on methamphetamine-induced dopaminergic neuronal damage: a positron emission tomography (PET) study with conscious monkeys

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Background: Previous PET studies of methamphetamine (METH) abusers suggested that psychotic symptoms may be attributable to the reduced dopamine (DA) transporters (DAT) in the human brain. To search the effective treatments, the neuroprotective effects of minocycline, a second-generation tetracycline, on METH-induced DA neuronal damages in the striatum (Str) were evaluated with animal PET with conscious monkeys. Aims: 1) To develop monkey model of impaired Str DA functions. 2) To assess the METH-induced damages of Str DA system with PET. 3) To evaluate the neuroprotective effects of minocycline on damaged Str DA system by METH.

Methods: This study included 10 male adult rhesus monkeys (Macaca mulatta, weight: 4.5 - 6.4 kg). To induce dysfunction of Str DA system, METH (2 mg/kg, i.m.) was injected three times with 3 hours intervals, and minocycline (200 mg/kg, s.c.) was administered 0.5 hr before first METH injection or 0.5 hr post 3rd METH injection in 1st day, followed by minocycline administration 2 times a day from 2nd day to 7th day. PET scans with ligands of [11C] - carbenoxolone -3 β - trifluoromethyl) imidazole -3β-propyl -5α - ([C] - CFT availability for DAT expression was analyzed. There was no significant difference in the scores of most of QOL dimensions after treatment. In 55% (6/11), suggesting a promising degree of clinical efficacy. There was no significant difference in the scores of most of QOL dimensions after treatment. In 55% (6/11), suggesting a promising degree of clinical efficacy.

Results: A total of 20 patients were entered in this study. The median number of courses delivered at the RD was 6, and the mean relative dose intensity of S-1 and CPT-11 was 0.97 and 0.96, respectively. Only 1 of 14 patients at the RD needed to reduce the dose of CPT-11. Eighty-five percent of the treatment combinations with the RD courses were delivered in the outpatient clinic. The MTD of CPT-11 was considered to be 100 mg/m2/day, because 2 of 3 patients developed DLTs, anorexia and diarrhea. Therefore, the RD of CPT-11 was set at the dose of 80 mg/m2. The overall response rate (RR) was 50% (7/14) and the RR at the RD was 55% (2/3), suggesting a promising degree of clinical efficacy. There was no significant difference in the scores of most of QOL dimensions after treatment. In general, the scores of QOL dimensions at 1 to 3 time point were not worse, and those at 4 to 6 time point were not better than those at baseline, respectively.

Conclusions: A combination of S-1 with CPT-11 can be recommended for further phase I studies in patients with advanced colorectal cancer.

Mechanism of Resistance in Non Hodgkin's Lymphoma- Expression of Fas Ligand on Endothelial Cells Lining Blood Vessels

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Background: In this study we investigated how the abnormal expression of Fas/FasL influences rituximab-mediated cell death, to establish modes of resistance in Mantle Cell Lymphoma (MCL) cells to current treatment regimens.

Methods: Xenograft models and an MCL-adipose injection of MCLZ-138 or JVM-2 cells into Rag-2mice. Rituximab is a monoclonal antibody targeted against the B cell marker CD20 and treatment at doses of 1, 2.5, 5 and 10 mg/kg was given every day for a total of 5 treatments in low tumor bearing mice (palpable) and high tumor bearing mice (200mg). Each treatment arm included groups of 6 mice and the study was done in triplicate. In vivo efficacy data was analyzed using SPSS software. The time taken for subcutaneous tumors to reach a size of 0.4 cm3 was analyzed using Kaplan-Meier curves for survival analysis. Treatment response and control groups were compared using a log-rank test. Patient samples were obtained from the BC Cancer Agency and consisted of 10 MCL, 10 Follicular Lymphoma, and 10 Diffuse Large B cell Lymphoma.

Results: In contrast to rituximab-insensitive JVM2 xenografts, tumors derived following injection of Z-138 human MCL cells were sensitive to rituximab, as judged by complete tumor regressions and decreases in cell proliferation (as measured by Ki67). Staining for Fas, in Z-138 xenografts demonstrates that Fast, is expressed on endothelial cells lining tumor blood vessels and was confirmed in patient samples. Coincidence of the FasL, signal and CD31 targeting blood vessels was confirmed by double immunofluorescence staining on frozen sections. Expression of Fas, lining tumor blood vessels led to exclusion of NK cells from the tumor microenvironment unless treated with Rituximab (as measured by immunofluorescence staining and real-time PCR). Tumor-associated macrophages were predominately in the tumor milieu and were downregulated following Rituximab treatment (as measured by immunofluorescence staining and western blot analysis).

Conclusions: 1) We describe here for the first time a novel mechanism of immune evasion in MCL and other Non-Hodgkin's Lymphomas via upregulation of FasL on endothelial cells lining tumor blood vessels.

Authors' disclosure statement: This work is currently unpublished and we trust that the data shared herein will remain confidential.
Interactions between Cytochrome P450, Cyclooxygenase and Nitric Oxide Synthase during Endotoxemia: Therapeutic Implications for Inflammatory Diseases


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**Background:** Increased production of nitric oxide (NO) and prostaglandins (PGs) associated with a decrease in formation of 20-hydroxyeicosatetraenoic acid (20-HETE) contributes to development of hypotension during inflammatory diseases such as endotoxemia. Aim: To summarize of our recent findings indicating interactions of cytochrome P450 (CYP) 4A, cyclooxygenase (COX) and inducible NO synthase (iNOS) mediate hypotension in rats treated with endotoxin (ET).

**Methods:** Conscious male rats received ET (10 mg/kg) or saline (4 ml/kg) at time 0. Blood pressure (BP) was measured using a tail-cuff device. Separate groups of ET-treated rats were given 1,3-PBIT (iNOS inhibitor; 10 mg/kg), indomethacin (nonselective COX inhibitor; 5 mg/kg), NS398 (COX-2 inhibitor; 10 mg/kg), 5,14-HEDGE (a synthetic analogue of 20-HETE; 30 mg/kg) or 20-HEDE (a competitive antagonist of vasoconstrictor effects of 20-HETE; 30 mg/kg) 1 h after injection of saline or ET. The rats were sacrificed 4 h after ET challenge and blood, kidney, heart, thoracic aorta (TA) and superior mesenteric artery (SMA) were collected for measurement of the enzyme protein levels and/or activities.

**Results:** ET-induced fall in BP were associated with increases in renal iNOS protein level and nitrite, 6-keto-PGF1α, PGF2α and TXB2 levels in the serum, heart, kidney, TA or SMA. ET decreased the renal CYP4A1 protein level/activity and the systemic and tissue levels of PGF1α, 1,3-PBIT prevented the ET-induced decrease in BP, renal CYP4A protein level/activity and increase in systemic and renal nitrite production. Indomethacin prevented the ET-induced decrease in BP, CYP4A protein level/activity, and increase in renal iNOS protein level/activity and systemic PGF2α production. 5,14-HEDGE, piroxicam or NS398 prevented the ET-induced changes in BP and systemic and tissue nitrite and eicosanoid production. These effects of 5,14-HEDGE were abolished by 20-HEDE.

**Conclusions:**

1. Certain isolated metal-quinolone complexes exert different kinds of antibacterial activity of metal complexes is not increased in comparison to that of free quinolones. The most interesting biological results were found for Cu-cfH complexes that are able to cleave DNA and for V-cfH-complexes that exerts in vitro insulin mimetic behavior. Interesting luminescence properties were also discovered for Cu-cfH complex.

2. Optical properties of europium-cfH complex might be useful for staining of tissues or for analysis of quinolones in biological samples.

3. Certain isolated metal-quinolone complexes exert different kinds of antibacterial activity of metal complexes is not increased in comparison to that of free quinolones. The most interesting biological results were found for Cu-cfH complexes that are able to cleave DNA and for V-cfH-complexes that exerts in vitro insulin mimetic behavior. Interesting luminescence properties were also discovered for Cu-cfH complex.

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**Acknowledgement:** This work was supported by USPHS-NIH Grant 19134-31, NIH Grant HLBI-19134-33A1, the Research Foundation of Mersin University (Project Code No: BAP ECZF EMB (BT) 2006-3), NIH Grant GM51278 and the Robert A. Welch Foundation.

Quinolones and Metal Ions: Friends or Enemies?

**TUREL I**

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**Background:** Unpredictable and never ending battle between bacteria and mankind shows that diseases considered to be controlled or even eradicated are appearing again, often in new forms that are multidrug resistant. Therefore it is extremely important to completely understand the molecular mode of action of existing drugs which could help us to exploit them even more efficiently in the future. In last years quinolones are clinically amongst the most successful synthetic antibacterial agents and one of the famous members of this large family-ciprofloxacin (cfH) is a real blockbuster drug. It is known that quinolones can in general easily interact with metal ions. In one hand metal-quinolone interactions are disturbing because the absorption of these drugs is reduced (due to the formation of sparingly soluble metal complexes). On the other hand it is believed that metal ions are needed for the biological activity of quinolones. The aim of this work was to prepare metal-quinolone complexes and study their properties.

**Methods:** We have isolated several novel metal ion (magnesium, copper, vanadium, bismuth, europium) -quinolone complexes and characterized them by various physico-chemical techniques (X-ray structure analysis, spectroscopy (IR, NMR, UV-Vis), etc.). Biological activity of complexes was also tested (antibacterial activity, DNA gynase activity, DNA cleavage, insulin mimetic behavior).

**Results:** Crystal structure of metal-quinolone complexes confirmed that bidentate (O, O') bonding of quinolone through ring carbonyl and carbonate oxygen atoms is predominant mode of coordination. Results have shown that antibacterial activity of metal complexes is not increased in comparison to that of free quinolones. The most interesting biological results were found for Cu-cfH complexes that are able to cleave DNA and for V-cfH-complexes that exerts in vitro insulin mimetic behavior. Interesting luminescence properties were also discovered for Cu-cfH complex.

**Conclusions:** Certain isolated metal-quinolone complexes exert different kinds of antibacterial activity of metal complexes is not increased in comparison to that of free quinolones. The most interesting biological results were found for Cu-cfH complexes that are able to cleave DNA and for V-cfH-complexes that exerts in vitro insulin mimetic behavior. Interesting luminescence properties were also discovered for Cu-cfH complex.

**All abstracts are listed in alphabetical order of the presenting author.**

**Abstracts**

**Page A-330**
Interaction between simvastatin, a HMG CoA reductase inhibitor, and grapefruit juice: in vitro characterization.

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Grapefruit juice is able to modify the pharmacokinetic parameters of many drugs. In particular, it increases oral bioavailability of simvastatin, a cholesterol lowering agent that acts by competitive inhibition of 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase. Simvastatin undergoes an important first pass metabolism and this is thought to be partly responsible for its low bioavailability after oral administration. Simvastatin is a prodrug that requires metabolic activation through hydrolysis by esterases to form the active simvastatin acid. Besides, simvastatin is a substrate for cytochrome P450 enzymes. It is also an OATP (organic anion transporter peptide) carrier substrate belonging to the solute carrier (SLC) family and MDR-1 efflux carrier belonging to the ATP binding cassette (ABC) superfamily of membrane transporters. The objectives of our work were first to identify some constituents of grapefruit juice involved in this interaction and secondly to characterize and quantify the mechanisms of this interaction in the intestine and in the liver. We have studied two groups of compounds involved in this interaction: (i) the flavonoids such as the nararingin and its aglycon form, the nararingenin and (ii) the furanocoumarins (psoralens) such as bergamottin and its metabolites. We have evaluated the effects of these two compounds (nararingin and bergamottin) on the intestinal transport and the intestinal and hepatic first pass metabolism of simvastatin by using different in vitro models. Our work has shown that these components of the grapefruit juice were able to modify absorption and metabolism of simvastatin by inhibition of CYP-450s (in particular CYP3A) and by modulation of ABC (MDR-1, MRP2) and OATP carriers. Thus, these constituents increased the bioavailability and plasma concentrations of simvastatin, raising its potential for adverse effects. These results should be taken into account to adjust doses in order to avoid adverse effects and risks of myopathy when simvastatin is co-administered with grapefruit juice.

A novel view of the pathophysiology of psychiatric disorders and development of pharmacotherapy based on brain energy metabolism

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Background: Lactate, an energy substrate for neural activity like glucose, has been shown to be produced by astrocytes under the regulation of glutamatergic tone. The serotonin (5-HT) system, specifically 5-HT1A receptors, is suggested to be involved in the modulation of lactate production. The majority of 5-HT1A receptors in the brain are found in several brain regions, which were blocked by pretreatment with WAY-100635.

Methods: 1) Tandospirone (2.0 mg/kg), a partial agonist at 5-HT1A receptors, and that 3) rapid energy demand induced by glutamate contributes to local lactate production, FS was administered. Artificial CSF containing DHK was perfused at a rate of 2.0 mL/min for 5 s administered every 30 s for 20 min) was administered using a plastic communication box connected to a shock-generator and a timer box. 2) Dihydrokainate (DHK), a glutamate uptake inhibitor, was dissolved in artificial cerebrospinal fluid (CSF). Artificial CSF containing DHK was perfused at a rate of 5.0 μL/min into the dialysis probe. Twenty minutes after the start of DHK (0.1 mM) perfusion, FS was administered.

Results: 1) Tandospirone attenuated the FS stress-induced increase of ELAC in both of the brain regions, which was blocked by pretreatment with WAY-100635.
2) DHK also attenuated stress-induced increase of ELAC in the mPFC, and completely blocked the lactate production increase in the basolateral amygdala (BLA).

Conclusions: The results of this study indicate 1) FS stress-induced increase in lactate production is partly regulated by 5-HT1A receptors both in cortical and limbic regions, 2) glutamate transporters regulate lactate availability in astrocytes, and that 3) rapid energy demand induced by glutamate contributes to local lactate production. Research into interactions between neurotransmitters and lactate metabolism may provide a novel view of the pathophysiology of some stress-related disorders, e.g., mood disorder, anxiety disorder, and post-traumatic stress disorder.

Authors’ disclosure statement: Part of this work was supported by Daninpon-Sumitomo Pharmaceutical Ltd. and Yoshinomi-yakuhin Ltd.
The critical role of IL-15 trans-presentation in the antitumor effects mediated by the combination therapy with Imatinib and IL-2

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Background: The synergistic antitumor effect of the combination therapy with imatinib mesylate (IM) and IL-2 has been shown to depend on NK1.1 expressing cells and led to the first description of an important effector cell population called Interferon producing killer dendritic cells (IKDC). IKDC were initially described as CD11c+ TcR- NK1.1 tumor infiltrating cells that mediated the antitumor effects of combination therapy with IM and IL-2 in B16F10 melanoma-bearing mice (Tabei et al. Nat. Med. 2000). This work aims at further investigating the mechanism involved in antitumor capacity of IKDC in this combination therapy.

Methods: We used a systemic mouse B16F10 melanoma model in C57Bl/6 mice and different transgenic mice. 3 x 105 B16F10 cells were injected at day 0 into the tail vein of mice. 200µl IM (Gleevec®, Novartis) 150mg/kg was given orally twice a day combined with IL-2 (1 x 10^5 IU of rhIL-2, twice a day i.p.) from day 0 to day 10 after tumor inoculation. Control groups received H2O/PBS, IM or IL-2 alone. At day 12, mice were sacrificed and lung metastases were enumerated.

Results: Here, we show that the antitumor efficacy of the combination therapy was compromised in IL-15 and IFN type 1R loss-of-function mice. IL-15R was required for the proliferation of IKDC during therapy with IM and IL-2. Trans-presentation of the IL-15 by IL-15R activated IKDC to express CCR2 and to respond to type 1 IFN by producing CCL2. Moreover, the antitumor effects of the combination therapy correlated with a CCL2-dependent recruitment of IKDC into tumor beds.

Conclusions: Our data indicate that the IL-15 driven peripheral expansion and intratumoral chemotaxis of IKDC is an important factor for the immunomodulatory effects of IM+IL-2.

Chemotherapy of Duchenne’s muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is a X-linked muscular abnormality caused by the loss of dystrophin and is one of the most genetically disorders. We have recently found that hematopoietic prostaglandin (PG) D synthase (H-PGDS) was induced in grouped necrotic muscle fibers in DMD patients (Okinaga T. et al., Acta Neuropathol., 104, 377-384 (2002)). Cyclooxygenase-2 and phospholipase A2 were also synchronously induced in the H-PGDS-expressing necrotic muscle fibers. We developed novel H-PGDS inhibitors based on the X-ray crystallographic analysis of human H-PGDS complexed with its prototype inhibitor, HQL-79 (Arante K. et al., J. Biol. Chem., 281, 15277-15286 (2006)). In this study, we investigated pathological significance of H-PGDS in a dystrophin null mdx mouse model and developed a novel therapy for DMD by inhibition of H-PGDS.

Methods: Localization of H-PGDS was examined in the dystrophic muscle fiber in a dystrophic null mdx mouse model by immunofluorescence staining with anti-mouse H-PGDS antibody. H-PGDS inhibitors were orally administrated to mdx mice for 5 days. The necrotic muscle in mdx mice was continuously measured by X-ray computed tomography (CT) imaging enhanced by non-ionic contrast media.

Results: H-PGDS was localized in the necrotic muscle fibers and accumulated macrophages in mdx mice. Oral administration of H-PGDS inhibitors prevented the expansion of muscular necrosis in a mdx mouse model and decreased the expression of mRNAs of pro-inflammatory cytokines. These results demonstrate that PGD2 produced by H-PGDS plays important pathological roles on the expansion of muscle necrosis. H-PGDS inhibitor also accelerated the accumulation and activation of macrophages in the necrotic area.

Conclusion: These results indicate that PGD2 produced by H-PGDS is involved in the expansion of muscle necrosis in DMD and that inhibition of H-PGDS is a novel therapy for DMD.

This study was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

Inhibition of Neutrophil Granule Exocytosis by a Novel Cell-Penetrating SNAP23 Fusion Protein: A Potential Magic Bullet?

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Background: Neutrophils are the primary cellular component of innate immunity. These cells contain four granule subtypes that undergo exocytosis in response to stimulation. The role of exocytosis in mediating neutrophil activation is unknown. The hypothesis that exocytosis contributes to specific neutrophil functional signatures was tested.

Methods: To inhibit exocytosis, fusion proteins containing the TAT cell permeability sequence and either the amino terminus SNARE domain of SNAP-23 (TAT-SNAP-23) or an inactive amino acid sequence (TAT-Control) were expressed. Transduction of TAT-SNAP-23 into neutrophils was confirmed by confocal microscopy. Exocytosis of secretory vesicles, specific granules and azurophil granules was determined using flow cytometry to measure the expression of CD35, CD66b and CDE3, respectively.

Results: TAT-SNAP-23 inhibited the FMLP-stimulated increase in CD35 and CD66b expression by 90% ± 7% and 75% ± 10%, respectively. Exocytosis of gelatinase granules, measured by release of gelatinase, was reduced by 60% ± 10%. TAT-Control had no effect on exocytosis of any of the four granule subtypes. TAT-SNAP-23 (5 µg/ml) reduced the stimulated increase in CD35 and CD66b expression by 90% ± 7% and 75% ± 10%, respectively. Exocytosis of gelatinase granules, measured by release of gelatinase, was reduced by 60% ± 10%. TAT-Control had no effect on exocytosis of any of the four granule subtypes. TAT-SNAP-23 (5 µg/ml) reduced the stimulated increase in CD35 and CD66b expression by 90% ± 7% and 75% ± 10%, respectively.

Conclusion: In conclusion, a fusion protein containing a cell penetrating peptide and a SNARE domain of SNAP-23 inhibits exocytosis of three of four neutrophil granule subtypes. The data show that granule exocytosis contributes to neutrophil chemotaxis and phagosomal respiratory burst activity, but not bacterial phagocytosis or respiratory burst activity stimulated by plasma membrane receptors.
Principal parallels between Interferon type I- and small inhibitory (si)RNA-based therapies

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Background: RNA interference (RNAi), a simple regulatory mechanism of gene expression and virus replication, was evolutionary replaced by the more complex interferon type I (IFN I) system. IFN I system is the major component of the innate immune response against viruses and part of the initial body defense against a range of microorganisms. However, it is less specific and it appears to be less efficient than RNAi against viruses. Here we will provide parallels between these two systems and discuss their advantages and disadvantages for the treatment of viral infections.

Methods: In this study primary cultures of peritoneal macrophages (mΦ) isolated from congenic flavivirus susceptible or resistant mice were used as a model to study various antiviral treatments against West Nile (WN) virus. The extent of antiviral effect of treatments with IFN I, polyinosinic/polycytidylic RNA (pIC) and several off-target (random) small inhibitory (si)RNAs was quantified by virus titration while the presence/absence of viral RNA was confirmed by RT-PCR.

Results: Untreated mΦ from both susceptible and resistant mice showed an initial 14% inhibition of virus growth. Following the priming with various antiviral agents, including the major antiviral cytokine IFN I, mΦ from resistant mice developed 2-4 stronger antiviral responses than cells from susceptible mice. However, a treatment with off-target siRNAs resulted in a complete eradication of WN virus replication in cells from both susceptible and resistant mice.

Conclusions: 1) Cells from resistant mice responded better to conventional antiviral treatments with IFN I and pIC than cells from susceptible mice. 2) Treatment with off-target siRNAs elicited the strongest antiviral effect in both cell types suggesting involvement of a resistance-unrelated mechanism. 3) Antiviral therapy based on small RNA may prove more efficient and direct than therapy based on IFN I.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Susceptible mΦ Virus titre (log10TCID50 units/106 cells)</th>
<th>%Percent inhibition (%)</th>
<th>Resistant mΦ Virus titre (log10TCID50 units/106 cells)</th>
<th>%Percent inhibition (%)</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>5.8</td>
<td>0</td>
<td>5.9</td>
<td>0</td>
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<tr>
<td>IFN I</td>
<td>4.9</td>
<td>16</td>
<td>2.0</td>
<td>66</td>
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<tr>
<td>pIC</td>
<td>3.2</td>
<td>45</td>
<td>0.8</td>
<td>86</td>
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<tr>
<td>siRNA</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
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</table>

*Percent inhibition relative to untreated mΦ from susceptible mice

When should we administer postoperative intra-peritoneal mitomycin therapy?

UZUNKOY A1, BOLUKBAS C2, HOROZ M3, BOLUKBAS F2, KOCYIGIT A2

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Background: There is controversy about the effect of the timing of intraperitoneal administration of chemotherapeutic agents on the healing of intestinal anastomosis. We have investigated the effect on intestinal wound healing of mitomycin-C administered at different times post-operatively.

Methods: Eighty-four Wistar-Albino female rats underwent ileal resection and end-to-end anastomosis. The rats were randomly selected for intraperitoneal administration of mitomycin-C or saline as follows: mitomycin-C group (n = 65), 2 mg/kg mitomycin-C; control group (n = 13), 10 ml saline. The former was sub-divided into 5 equal groups (A-1-5) and mitomycin-C was administered postoperatively as follows: day 0 (A1), day 3 (A2), day 5 (A3), day 7 (A4) and day 10 (A5). All the rats were sacrificed on the 14th postoperative day and anastomotic bursting pressures and tissue hydroxyproline levels were determined.

Results: Five of the animals died postoperatively: 2 (15.4%) in group A1, 2 (15.4%) in group A2 and 1 (7.7%) in group A3. Non-lethal anastomotic leakage was observed in a further five animals: 1 in group A1, 2 in group A2, 1 in group A5 and 1 in the control group. Groups A1 and A2 had significantly lower anastomotic bursting pressures than the other groups (p < 0.05 for each comparison). The anastomotic bursting pressures of group A3, A4 and A5 were comparable with those of the controls (p >0.05 for each comparison). Tissue hydroxyproline levels in group A1 and A2 were significantly lower than in the controls (p values were <0.05 for each comparison) or the other mitomycin-C sub-groups (p = <0.05 for each comparison).

Conclusions: Intraperitoneal chemotherapy impairs intestinal wound healing when applied before the 5th postoperative day. Additional therapeutic approaches are needed to prevent this potentially lethal side effect of early intraperitoneal mitomycin-C administration.

Authors’ disclosure statement: This study has been published BMC Cancer as ‘the optimal starting time of postoperative intraperitoneal mitomycin-C therapy with preserved intestinal wound healing’.
Commercioazional of Recombinant Human Epidermal Growth Factor – A Nobel Prize Winning Molecule with Diverse Therapeutic Applications

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* Author for correspondence

Background: Growth Factors are a distinct class of signaling proteins that modulate wound healing at a molecular and cellular level. One such Growth Factor is the Epidermal Growth Factor (EGF), a 6.2 kDa protein, whose discovery by Prof Stanley Cohen got him the Nobel Prize along with Dr Rita Levi-Montalcini in 1986. The role of EGF has been extensively investigated in normal and pathological wound healing and EGF based formulations are currently gaining therapeutic importance in wound management. Such formulations, for obvious reasons, are developed using recombinant human EGF (rhEGF). Presentation Reviews the various approaches adopted to produce rhEGF on a large scale including the work carried out by the Authors’ Group. We have been successful in producing rhEGF at a commercial level through a cost-effective, novel process yielding >95% pure active protein with high specific activity.

Methods: rhEGF and formulations thereof: Several studies have been reported earlier on cloning in E. coli and yeast system for the expression of recombinant human EGF and purification by RP-HPLC and Affinity Chromatography. A serious limitation of most of these studies is the first yield (~100 mg), and the purity (~85%), thus limiting the commercial potential of such work. In our objectives towards commercialization of rhEGF, we have expressed rhEGF as a tetramer or a trimer protein consisting of N terminal TGF sequence and C terminal with six arginine residues attached to the human EGF sequence. This basic gene was cloned in HT1113, a strain under different conditions in a Bioreactor and was found to be a potential host with high transfection and productivity. The bioreactor conditions were optimized to express the protein as an inclusion body which was estimated around 350mg, and the yield obtained after renaturation purification was about 450mg. Some of the novel aspects of our work include: production at E. coli strain HT1113 for the expression of recombinant human EGF, and purification by RP-HPLC. Size exclusion chromatography, MALDI-TOF, Circular Dichroism, N-terminal sequencing and the formation of tetramer were assessed by ELSA using functional monomeric antibodies and 3D CD line. rhEGF based Therapeutic Formulations. Extensive formulation development work (both Cream and Gel based formulations) was done to make stable and suitable Formulations at 3 different pH levels for various applications. Safety of such Formulations has been evaluated by in-depth investigations in healthy volunteers. We have tested rhEGF in various formulations, including topical, injectable, oral, and transdermal delivery systems for the expression of recombinant human EGF and formulations based on this work.

Conclusion: This Presentation describes the successful large scale manufacture of rhEGF and formulations based on this work for enhanced wound healing in diverse cases involving diabetic foot ulcers.

New Drug Development Outside of G8: Ready, Shoot, Aim

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Background: Clinical assessment in late-stage drug development should be guided by results of comprehensive preclinical safety assessments and rigorous clinical pharmacology programs. In South America (SA), new drug development is generally conducted at public universities without the support of local pharmacology companies willing to participate in a government-supported research project. However, for safety reasons, SA pharmacists are trained to work with physical-chemical principles by RP-HPLC. Size exclusion chromatography, MALDI-TOF, Circular Dichroism, N-terminal sequencing and the formation of tetramer were assessed by ELSA using functional monomeric antibodies and 3D CD line. rhEGF based Therapeutic Formulations. Extensive formulation development work (both Cream and Gel based formulations) was done to make stable and suitable Formulations at 3 different pH levels for various applications. Safety of such Formulations has been evaluated by in-depth investigations in healthy volunteers. We have tested rhEGF in various formulations, including topical, injectable, oral, and transdermal delivery systems for the expression of recombinant human EGF and formulations based on this work.

Conclusions: This Presentation describes the successful large scale manufacture of rhEGF and formulations based on this work for enhanced wound healing in diverse cases involving diabetic foot ulcers.

Lactoferrin Acts Against Infection and Inflammation through its Influence on Systemic Iron Homeostasis

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Background: Infections and infections are often associated to hypoferremia. Hypoferremia is an important signal of disorders of hepatic iron levels which regulate the entry of iron into plasma and the liver as ferritin. The production of infection-modulating cytokines is difficult to control. The overall goal is to modulate the systemic iron homeostasis through the decrease of serum IL-6, key cytokine in the iron management. Based on this concept, we hypothesized that a) Lf exerts a potent effect in restoring the iron transport from cells to circulation and b) Lf can be used as a novel therapeutic agent against infections.

Methods: The clinical trial on the therapeutic effect of Lf on systemic iron homeostasis included 171 subjects, suffering of hypoferremia. Subjects were randomly divided in two groups. The first group received an oral administration of 520 mg of ferrous sulphate, once a day (156 mg as elemental iron), and the second group received orally 100 mg of Lf, twice a day (8.8 mg as ferric iron).

Results: Haematological values and IL-6 concentration were assessed on venous blood.

Conclusions: Lf exerted a potent effect in restoring the iron transport from cells into circulation through the decrease of serum IL-6 concentration which in turn modulates hepatic and foetal synthesis. In contrast to the administration of ferrous sulphate, Lf oral administration did not result in any side effect. The capacity of Lf to decrease IL-6 could represent a novel therapeutic alternative in reduction of host inflammation and susceptibility to infections.
Molecular Characterization of Antibiotic Resistance in Selected Enteropathogens Isolated from Raw Food Samples in Vietnam

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1RMIT University, Melbourne, VIC, Australia; 2University of Natural Sciences, HCM City, Vietnam

Background: The emergence of antibiotic resistance in bacteria has become a serious problem worldwide. This study aims to determine the molecular characteristics of antibiotic resistance in enteropathogens isolated from raw food samples in Vietnam.

Methods: Raw food samples (n=180, comprising meat, poultry, and seafood) were collected from Ho Chi Minh City, Vietnam for the isolation of Salmonella spp., Escherichia coli and Vibrio parahaemolyticus. The isolates were tested for antibiotic resistance against 15 commonly used antibiotics by the disk diffusion method. They were further examined for the presence of mobile genetic elements conferring antibiotic resistance. Transfer of antibiotic resistance phenotypes was studied by conjugation. Salmonella genomic island 1 (SGI1) antibiotic resistance gene cluster was investigated using PCRs, Southern blot analysis and sequencing.

Results: E. coli and Salmonella spp. were isolated in 60.8% and 48.9% of the samples respectively. V. parahaemolyticus was present in 32% of shellfish samples. There were high antibiotic resistance frequencies in E. coli and Salmonella spp. isolates, especially to tetracycline, ampicillin, nalidixic acid, streptomycin, and sulphonamide. E. coli also showed high resistance to trimethoprim (43.4%) and chloramphenicol (51.5%). Multi-resistance, i.e. resistance to at least three different classes of antibiotics, was detected in 61.6% E. coli and 20.9% Salmonella isolates, including potential human-pathogenic Salmonella serovars. V. parahaemolyticus isolates were uniformly resistant to ampicillin. Integrons harbouring genes responsible for resistance to aminoglycosides, ampicillin, trimethoprim and chloramphenicol were found in 57% E. coli and 13% Salmonella spp. isolates. Plasmids were detected in all tested isolates, many of them were larger than 95 kb. Antibiotic resistance phenotypes were found to be transferable among the isolates. SGI1 was identified in Salmonella sewerov Albany isolated from chicken meat.

Conclusions: The results indicate that raw foods of animal origin in Vietnam are potential reservoirs for multi-resistant pathogenic organisms which contain a pool of mobile genetic elements, raising the awareness of antibiotic resistance in food pathogens.

Passive Immunization for the Protection of our Global Society against Emerging Infections

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Background: New viral diseases, like the Severe Acute Respiratory Syndrome (SARS), are an enormous threat to our society. With more than 50% of the global population living in cities and 2 billion airline passengers per year, there is no way to escape from contact with infected persons once a pandemic starts. The World Health Organisation (WHO) devoted the World Health Report 2007 to this topic, which concludes that there will be another Ebova or SARS sooner or later. Vaccination would be the preferred way to protect the population, but the development of a new vaccine takes about 10 years. Therefore serum therapy is a serious candidate for the therapy and post exposure prophylaxis of new viral diseases.

Analysis: There are several powerful approaches to generate and produce humanized or human monoclonal antibodies. But production in mammalian cell lines in bioreactors is still an expensive process and at this moment there is insufficient production capacity to produce the required quantity of antibodies.

Solutions: Innovation in production technology is necessary. One solution could be to use plants for the production of the monoclonal antibodies. Another way to solve the problem would be to make more potent neutralizing antibodies than can be administered at lower concentrations. In view of the importance of protection of the global population and the lack of alternatives, a task force for the necessary innovation is needed.

One target, two bullets: from erythromycin to telithromycin, what makes the difference?

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Background: the increasing threat of bacterial resistance and the progress of modern pharmacology stimulate the search for antibiotics capable of bypassing existing resistance mechanisms and presenting an optimized pharmacological profile.

Methods: overview of the rational design of telithromycin (TEL), as first clinically-used ketolide and semi-synthetic derivative of the macrolide erythromycin (ERY), in relation with its novel pharmacological profile.

Results: SAR: TEL differs from ERY by (a) removal of the cladinose at position 3 and its replacement by a ketoo-group, (b) incorporation of an 11,12- cyclic moiety, and (c) addition of a heteroaryl-alkyl side chain. TEL can therefore interact with 2 distinct binding sites in the 23S rRNA of the ribosome. This increases its activity against ERY-S strains and maintains activity towards ERY-R S. pneumoniae with ribosomal methylation. TEL is also a poor inducer of methylase expression and less susceptible to efflux in S. pneumoniae.

PK: TEL shows an improved PK profile, with high oral bioavailability (improved AID stability), penetration in tissues and fluids, accumulation within eucaryotic cells and poor recognition by eucaryotic efflux transporters (P-glycoprotein), prolonged t1/2 allowing for daily administration.

PD: PK/PD considerations suggest optimal efficacy for isolates with MICs ≥ 0.25 mg/L. As ERY, however, TEL is bacteriostatic in vitro and poorly active in models of intracellular infections despite its high cellular accumulation.

Safety: TEL causes less gastrointestinal side effects and is a less potent inhibitor of CYP3A4 than ERY. It causes however rare but serious side effects (hepatotoxicity, respiratory failure in patients with myasthenia gravis, visual disturbance, risk of QTc prolongation).

Conclusions: this story tells us how knowledge of targets helps in obtaining more powerful bullets. Further optimization of PD and safety is still needed to get "the" magic bullet acting on bacterial ribosomes.
Pharmacodynamic Monitoring of Calcineurin Inhibition Therapy

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Introduction: Calcineurin inhibitor (CNI) therapy by cyclosporine or tacrolimus is still considered the backbone of immune suppression after allograft transplantation, especially early after transplantation. Since both CNIs show unpredictable pharmacokinetic profiles, TDM is used to control drug exposure. Unfortunately, CNI therapy is still associated with severe side effects such as nephrotoxicity and unfavorable cardiovascular risk profile. To optimize CNI therapy, more accurate monitoring strategies are required and a pharmacodynamic strategy constitutes a novel and innovative approach.

Content: Several pharmacodynamic monitoring strategies have been developed that all showed inverse relations between CNI concentration and pharmacodynamic markers, such as calcium (CNi) activity and T cell activation transcripts. These markers have been investigated for allograft recipients treated with CNIs and although the first signs of their usefulness have been reported, they are not suitable for routine monitoring so far. A feature of many pharmacodynamic monitoring strategies is that the large inter-individual variation observed does not necessarily explain clinical and immunological observations. We have investigated whether sample choice and composition could contribute to the observed large inter-individual variation of these markers. We have therefore monitored sample composition when either total leukocyte fraction or peripheral blood mononuclear cells (PBMCs) are chosen as sample in renal allograft recipients. Next cellular CNi activities and CNi inhibition profiles for cyclosporine were determined. We found that based on the differences in cellular CNi activity, the large variation in sample composition could indeed confound measurement outcome. Cell-specific CNi activities and drug sensitivity should therefore be considered for sample validation.

Conclusions: The call for more accurate monitoring strategies for immunosuppressive therapy has led to the development of a large variety of pharmacodynamic monitoring strategies. The first indications of their clinical relevance are available, but further understanding of the analytical and clinical variables involved seems to be required before these markers can be tested in a clinical setting.

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The regions of antigens that are recognized by antibodies are called epitopes while the regions of antibodies that recognize epitopes are called paratopes. Most epitopes of proteins are called discontinuous epitopes because they arise from between two and five separate segments of the polypeptide chain, that are brought together by the folding of the chain. These epitopes exist only because the chain acts as a scaffold and if the scaffold is perturbed, the epitope ceases to exist. It is not possible to isolate such epitopes independently of the rest of the protein in which they are embedded in order to show that they possess binding activity on their own. It has also not been possible so far to predict them or to reconstruct them by synthesis since this would require assembling atoms which in the protein are not held together by internal chemical bonds. Most residues at the surface of a native protein contribute atoms to a large number of overlapping epitopes recognized by different antibodies. No clear boundaries exist between neighboring epitopes which together form a series of antigenic sites and it is only because Mabs are used as analytical tools that protein antigenicity appears to be located in discrete epitope regions rather than in an antigenic continuum. Unfortunately the strategy of focusing on individual epitopes has been detrimental to the search for effective vaccine immunogens. Proteins are also said to possess continuous epitopes, defined as linear protein fragments of 5 - 15 residues that are able to bind to antigen antibodies. Many of the residues of such continuous epitopes are not present at the surface of the parent protein and these epitopes are thus poor structural mimics of the antigenic regions of native proteins. It is only because the antibodies are poly specific and able to cross-react with short peptides, consequently to separate segments of complex discontinuous epitopes, that large numbers of continuous epitopes have been described. It is often mistakenly assumed that it is justified to extrapolate from cross-reactive antigenicity (binding of peptides to antiprotein antibodies) to cross-reactive immunogenicity (raising antipeptide antibodies able to cross-react with the cognate protein). However, the structure of an epitope determined when it is complexed with a paratope tends to differ from the structure present before the process of mutual adaptation that occurs when the two binding partners interact. The structure of an epitope after complexation with a neutralizing Mab is therefore an unreliable guide for defining which vaccine immunogens are needed to elicit protective neutralizing antibodies.

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Abstracts

The Role of the Dopamine Transporter Gene in Smoking and Other Addictive Behaviors

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Background: Genetic analysis of the human dopamine transporter (DATAO or SLC6A3) has been a central research focus since the cloning of the gene and identification of sequence variants in 1992. The most frequently assessed site is a Variable Number of Tandem Repeats (VNTR) present in the 3'-untranslated region of the gene. Assessment of smoking and addiction-related traits that have a genetic component is made difficult due to the complex nature of these phenotypes. A scan of the literature suggests that new approaches to genetic analysis of smoking, which is the focus of this presentation are needed.

Methods: We analyzed gene-by-environment (G X E) interactions in an intervention program. Children selected at 3 or 6 years old based on ratings of difficult behavior in the classroom were given interventions including training for individual behaviors, interactions with peers, and parent-child interactions. A control group from the same schools, but without specific interventions, was used for comparison. Assessment of smoking and alcohol use in school grades 9, 10, and 11 was used as the outcome variables. There were 1199 participants of which 959 were successfully genotyped, resulting in a group that was 63% male, 48% European-American, 48.6% African-American for this study. The VNTR genotype was assigned as either 9-copy present or absent. Logistic regression was used to test main effects of HDAT. Those individuals that possess the 9-copy allele and given the intervention had lower levels of smoking and alcohol use.

Results: The present G X E study showed significant effects of the combination of genotype and intervention on drug use, while little of significance was seen for the main effect of HDAT. Those individuals that possess the 9-copy allele and given the intervention had lower levels of smoking and alcohol use.

Conclusions: Published results have been mixed as to any consensus for a role for variants of HDAT in smoking and other addictive behaviors. Our G X E study showed significant effects suggesting that interactions might have larger consequences than main effects of the genes themselves.

The natural steroidal withanolide Withaferin A is a novel promising chemosensitising compound in B-cell chronic lymphocytic leukemia and metastatic breast cancer


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Background: B-chronic lymphocytic leukemia (B-CLL), the most prevalent leukemia in adults in Western countries, still remains incurable. In aggressive metastatic breast cancer cells, loss of hormone receptors frequently leads to hormone therapy resistance.

Aims: We wanted to investigate whether Withaferin A (WA), a natural steroidal withanolide purified from Withania somnifera (Ashwagandha), is able to induce apoptosis in B-CLL and hormone refractory metastatic breast cancer cells and we explored the molecular mechanisms involved.

Methods: We determined IC50 values of WA in B-CLL samples and metastatic breast cancer cells and evaluated various markers of apoptosis. Furthermore, we studied effects of WA on NF-κB/Stat3 signaling pathways involved in B-CLL and breast cancer survival.

Results: WA induced apoptosis in B-CLL and metastatic breast cancer cells with IC50-values of 0.60±0.28 and 50±25 µg/ml respectively. Cell death was associated with impaired NF-κB activation and increased protein ubiquitination levels, independently of 20S proteasomal inhibition. This correlated with selective downregulation of various B-cell growth factors and anti-apoptotic gene products, including Il-6, Il-8, Xiap, A1m38, Bcl-xI. In addition, we found that anti-invasive activity of WA results from cumulative inhibition of constitutive and inducible regulatory steps in the canonical and noncanonical NF-κB-signalling pathways. This allows WA to potently repress expression of interleukin (IL6, IL8) and metalloproteinase (MMP3, MMP9) survival factors.

Conclusions: These data illustrate the potential of WA as a multi-targeted NF-κB/Stat3 inhibitor and novel promising chemosensitising compound in B-CLL-and breast cancer therapy.
A new “quasi-adaptive” response to alkylating agents in E. coli cells due to posttranslational modification in S-nitrosylated Ada protein. Igor VASILEVA SV Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia

Abstract: An experimental verification of the original hypothesis - a "quasi-adaptive" response (quasi-Ada) to alkylating agent in E. coli. Assessment perspectives of a new phenomenon application in medicine since structure and functional relationship of E. coli Ada genes and their mammalian counterparts. Aims: 1)To study if S-nitrosylation (instead of S-alkylation in "true Ada") of the key target in Ada protein might serve the signal for the quasi-Ada activation and contribute to the adapted cell resistance to challenge nitrosomethylene (NNM). 2)To answer the question if NO donor structure will affect the level quasi-Ada induction 3)To establish the structure of NO signal molecule. 4)To develop optimized protocol for NO donor application as pseudo substrate in experimental chemotherapy. Methods: A collection of E. coli mutants bearing aakI::lacZ; aakII::lacZ and aadI::lacZ operon fusion [Volkert, 1998] was used. Dinorilisoyl iron complex (DNCu2+) [Varrin, 1996] and a new stable thiorhodol complex iron complex with thiourea (TNICu) [Sanina, Aldoshin, 2000] were studied as NO donating agents. EPR-resonance spectroscopy was applied to detect a formation inside the cells the typical anisotropic EPR signal with g-factor 2.03. To measure the concentration of NO molecules generated we used an ami-N0-700 sensory electrode from in NO NitrOxide Measuring System. Results: Quasi-Ada induction by low toxic DNCu2+ could be quantified by 3-5 fold increasing in the levels of aakI and aakII expression and 1.5-2.5 fold decreasing in the rate of mutations and lethal lesions, induced by NMM. TNICu was selectively effective in the aakI gene activation and in inhibition of the aakI and aadI genes expression. Intracellular iron was indispensable for NO-signaling: o-phenoaniline (OP) already prevented the phenomenon. Treatment of the cells with NO donors led to formation of the EPR signal with g 2.03, which disappeared after OIP cell pretreatment. It appeared that namely [Fe(NO)2]3 - mediated signaling cascade extended to the level of the Ada-gene transcription. In NMM responsive human tumour models of different genetic TNICu increased their NMU sensitivity up to 36-62%. The promising results were obtained with some new NO-donors as the "independent" drugs, as well. Conclusions: The new quasi-Ada phenomenon extends NO functions in genetic signal transduction within the Ada response system. E. coli can be used as a valid model in identification of the new promising regulators of adaptive processes in mammals. This phenomenon is currently under study. This work was supported by Russian Foundation for Basic Research (08-04-00228).

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The Prognostic Value of Genetic and Phenotypic Markers of Drug Metabolism and Host Exposure Factors for Antibacterial Drug Induced Hepatotoxicity

VAVILOV VA1, MAKAROVA SI2, KOLPAKOV TA2, KUDRISHEVAV2, MUKHAMBETJAN2, NIKISHINA MI1, KULIKANOVA LA1, POLYANSKIYA LV3, KRAISOV VA1, LYAKHOVICH VV1

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Background: The intensive antibacterial treatment regimens have as a concomitant result the high frequency of adverse reactions involving hepatotoxicity. Estimation of patient individual genetic features in antibacterial drugs metabolism assume as a perspective approach to decreasing risk of these reactions. However discordance of genotype and phenotype in drug metabolism is possible.

Methods: We investigated the possibility of the hepatotoxicity prediction in patients receiving isoniazid, rifampicin, pyrazinamide and ethambutol on the basis of isoniazid pharmacokinetics and genetic polymorphism of N-acetylationtransferase 2 enzyme. It was detected 282C>T, 481C>T, 590G>A, 803G>A and 857G>A SNPs in 75 patients with lung tuberculosis and in 52 patients was detected transferred pharmacokinetics of isoniazid in first days of therapy.

Results: Distribution of pharmacokinetic parameters of INH elimination (constant of elimination, Ke; clearance total (Cl) and time of half-elimination (t1/2) was bimodal with antimodes 0.2 h-1; 3.5 min/mg and 3.2 h, respectively. Median values of t1/2 were equal 6.4 h for slow and 1.8 h for rapid acetylators. Frequencies of 481T, 590A and 857A alleles were 0.36, 0.29 and 0.60, respectively. In examined patients were 13 cases (26%) of inconsistency between genetically predicted and pharmacokinetically determined acetylator phenotype, 11 of them were dislocation of genetically fast acetylators into phenotypically slow acetylators. Level of urine acetoacetate/creatinine (ALT) was elevated during first month of therapy in genetically as well as pharmacokinetically determined slow acetylators. However level of statistical significance of differences between initial values of ALT with those after 1, 2 and 3 month of therapy was higher in analysis with pharmacokinetic data.

Conclusions: The pharmacokinetic estimations are more preferable for individual prognosis of drug-induced hepatotoxicity.

Physico-Chemical Insights in Biological Conversions; the Role of H2O as driving Force in Cytochrome P450 catalysis

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Background: By far many of the involvement of the oxenoid iron form (PorFe=O) as active component in cytochrome P450 catalysis was generally accepted by many open questions.

Method and Conclusions: An alternative approach was made by the study of microperoxidase-8 (MP-8). MP-8 is a peptide of eight amino acids bound by two cysteines to the pore ring of the heme cofactor and ligated to a histidin of the peptide chain. MP-8 acts as a peroxidase in the presence of H2O2 that is fully inactivated by the addition of ascorbate, but remains active in different P450 activities. In the peroxidase mode it catalyses rather complicated polymerization of organic substances

Conclusions: The pharmacokinetic estimations are more preferable for individual prognosis of drug-induced hepatotoxicity.

Unprecedented Antitumor Effect of Irradiation Generated by 5-Trimethylsilyl-2-Trifluoroacetylfuran Oxime in Fibrosarcoma Cells

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Background: Numerous experiments evidence that spontaneous or artificially induced signalling photon emission (SPE) produced by unicellular and multicellular organisms serves not only for the regulation of metabolism but also for their biocommunication with surrounding biological systems. Emission, trapping, modification and other aspects of SPE are mainly confined in the boundaries of biological system. However its small part break loose in outer space and could be detected both qualitatively and quantitatively using appropriate physical equipment or detectors of biological origin. During antitumor screening of organic substances in vitro using monolayer cells, we unexpectedly found that SPE with strong cytotoxic effect could be induced by 5- trimethylsilyl-2-trifluoroacetylfuran oxime (OS-8596).

Methods: The solution of IOS-8596 was added in 3 rows of conventional polystyrene 96-multwell plate containing monolayer human fibrosarcoma cells (HT-1080). Thus prepared plate was cultivated at 37°C for 72 hours. A quantitative assay for the cell population treated and untreated with IOS-8596 was performed by staining with crystal violet (CV) or Coomassie Brilliant Blue R-250 (CB)

Results: It was found, that IOS-8596 killed the cells, not only in the well-emitters in which it was added, but also in adjacentwell-detectors containing cell culture alone. The intensity of cytotoxic effect observed in well-detectors directly depended on the amount of IOS-8596 added in well-detectors. The addition of -carotene in well-emitters. The addition of -carotene in well-detectors or in well-emitters resulted in 2-fold increase of cytotoxic effect in well-detectors compared with the control.

Conclusions: Thus obtained experimental data evidence about the development of cytotoxic effect in well-detectors compared with the control. The Prognostic Value of Genetic and Phenotypic Markers of Drug Metabolism and Host Exposure Factors for Antibacterial Drug Induced Hepatotoxicity

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Results: Distribution of pharmacokinetic parameters of INH elimination (constant of elimination, Ke; clearance total (Cl) and time of half-elimination (t1/2) was bimodal with antimodes 0.2 h-1; 3.5 min/mg and 3.2 h, respectively. Median values of t1/2 were equal 6.4 h for slow and 1.8 h for rapid acetylators. Frequencies of 481T, 590A and 857A alleles were 0.36, 0.29 and 0.60, respectively. In examined patients were 13 cases (26%) of inconsistency between genetically predicted and pharmacokinetically determined acetylator phenotype, 11 of them were dislocation of genetically fast acetylators into phenotypically slow acetylators. Level of urine acetoacetate/creatinine (ALT) was elevated during first month of therapy in genetically as well as pharmacokinetically determined slow acetylators. However level of statistical significance of differences between initial values of ALT with those after 1, 2 and 3 month of therapy was higher in analysis with pharmacokinetic data.

Conclusions: The pharmacokinetic estimations are more preferable for individual prognosis of drug-induced hepatotoxicity.
Vulvovaginal Colonization by Aspergillus Species in Nonimmunocompromised Women

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This study was undertaken to determine the prevalence of female genital Aspergillus infections. Additionally, the study explored whether genital Aspergillus infections are associated with Aspergillus infections elsewhere (e.g., pulmonary, cardiac, orthopedic, or ophthalmologic). Between October 2005 and October 2007, vulvovaginal fungal cultures were performed for all patients seen in the Vulvar Disorders Center at the Good Samaritan Hospital, Cincinnati, OH (n=351), and at Wright State University-Boonshoft school of medicine department of Obstetrics and Gynecology (Dayton, OH). Prospectively, any cultures showing Aspergillus species were segregated and a running list totaling 16 patients was maintained. The prevalence for Aspergillus in the vaginal culture was 6 per 1,000. The patients were all followed, examined and cultured at each subsequent visit for genital and extragenital disease. The patients responded to therapy with a culture-proven elimination of the fungus. Splanchnospor (Inonocarospor; Jansens Pharmacueticals, Titusville, NJ) 200 mg daily for 30 days proved to be the most objectively effective regimen for the treatment of high blood pressure (BP), other CV diseases and renal dysfunction. The CV and renal actions of DA and dopaminergic compounds are dose-dependent. In some types of hypertension, DA is known to influence the control of arterial BP by influencing the central and peripheral nervous system and target organs such as the kidneys and adrenal glands. DA and DA-agonists, including inhaled DA have a role in the treatment of high BP, other CV diseases and renal dysfunction. Hence, it is important to review the physiological and pharmacological aspects of DA and its receptors, and the potential clinical utility of DA and its derivatives in the therapy of hypertension, other CV diseases, and renal dysfunction.

Conclusion: DA is an important neurotransmitter with varied physiological action in the CV and renal systems. DA and DA-agonists, including inhaled DA have a role in the treatment of high BP, other CV diseases and renal dysfunction.

Preparative chemoradiation with concurrent Capcitabine for Locally Advanced Rectal Cancer (LARC): A Phase II study

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Background: A prospective one-arm phase II study was performed between 6/2004-1/2005 to assess efficacy and toxicity of preparative radiotherapy (RT) and concurrent oral capcitabine in patients with LARC.

Methods: Patients were irradated to 45 Gy in 25 fractions over 5 weeks to the pelvis concomitantly with oral capcitabine 825 mg/m2 bid including weekends. Surgery was scheduled 4-6 weeks after completion of the chemoradiation. Four courses of chemotherapy were planned postoperatively. The primary endpoint of the study was complete response rate; secondary endpoints included toxicity, survival and long-term rectal and urogenital morbidity. Patients still alive and without recurrence of the disease, with a minimum follow up of 1 year, were questioned with LENT/SOMA late-effect scale for rectum, bladder and sexual function.

Results: Fifty-seven patients entered the study (median age 67 years, 43 males and 14 females). During preparative part, one female died after receiving 27 Gy (pulmonary embolism) and the most frequent grade 3 toxicity was dermatitis (33.9% of patients). Radical operation was performed in 55/67 of patients. The complete pathological response rate was 9.1%, T-, N- and overall downstaging rates were 40%, 52.9% and 49.1%, respectively. A total sphincter preservation rate was 85.6%. During the early peroperative period, one patient died due to sepsis. Five patients (10%) were reoperated due to anastomotic leakage, intraabdominal abscess, ileus, enterocutaneous fistula and stomal occlusion. At 1 year of follow-up, the rate of patients with severe late (SOMA grade 3 and 4) rectal, bladder and sexual toxicity was 40%, 19.2% and 31%, respectively. The local relapse has occurred in 1 (1.8%; 13/46) and discontinuation of treatment in 1 patient due to toxicity at 19.8 weeks. At 1 year of follow-up, metastasis developed in 4 patients (7.0%). At median follow-up of 23 months (range 3-23 months). Second primary malignancy has occurred in 2 patients. The actuarial 2-year (median follow-up) OS, DFS and DSS rates were 84.2%, 72.5% and 92.4%, respectively, and local control was 98.2%.

Conclusions: Preparative chemoradiotherapy with oral capcitabine is well tolerated, safe and effective treatment regimen of LARC. It results in excellent local control. The rate of distant relapse and late functional morbidity is still of concern and asks for new treatment approaches.

Hyaluronan Mixed Esters of Butyric and Retinoic Acids: Multicomponent-Multitarget Drugs with Differentiating and Paracrine Logics for Cardiovascular Repair with Human Mesenchymal Stem Cells

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Cell lineage specification is factionalized at multiple intercrossed levels and is controlled by a complex interplay between cell signaling, microenvironmental assembly, the establishment of multilayered transcriptional networks, and the activities of embryonic and regenerative stem cells. Despite major advances in regenerative medicine in recent years, the clinical application of autologous hematopoietic progenitor cells for the treatment of hematologic and solid malignancies remains limited. We applied two factors, HA and HBR, to repair infarcted myocardium, suggesting that HB R-treated cells may also contribute to neovascularization and heart rescue through their ability to generate capillary-like structures. Some of the transplanted cells that had been pretreated with HBR also expressed vWF-positive cells detected in samples from the untreated group. A consistent organization of human vWF positive cells FMhMSCs, the yield of cells positively stained with a human-specific anti-vWF antibody remarkably exceeded the number of FMhMSCs may have contributed to the observed cardiac repair. Besides this, in the hearts injected with HB R-exposed xenogeneic FMhMSCs, the yield of cells positively stained with a human-specific anti-vWF antibody remarkably exceeded the number of FMhMSCs, the yield of cells positively stained with a human-specific anti-vWF antibody remarkably exceeded the number of FMhMSCs, the yield of cells positively stained with a human-specific anti-vWF antibody remarkably exceeded the number of FMhMSCs.

HBR treatment in the hearts of rats subjected to acute myocardial infarction led to complete normalization of pathological remodeling and significant improvement in cardiac function and morphology. Moreover, HBR-pretreated stem cells may contribute to heart rescue through the ability to generate capillary-like structures. Some of the transplanted cells that had been pretreated with HBR also expressed vWF-positive cells detected in samples from the untreated group. A consistent organization of human vWF positive cells FMhMSCs, the yield of cells positively stained with a human-specific anti-vWF antibody remarkably exceeded the number of FMhMSCs, the yield of cells positively stained with a human-specific anti-vWF antibody remarkably exceeded the number of FMhMSCs.
Newer antifungal drugs in kidney transplant recipients

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Background: Infection is a common cause of morbidity and the second cause of death in cardiovascular disease in renal transplant recipients. Although renal transplant recipients have been thought to be at low risk for fungal infections compared with other transplant recipients, the rate of hospitalization for fungal infections is much higher than the general population. Aspergillosis in renal transplant patients is the commonest cause of systemic fungal disease with an incidence ranging from 0.4 % to 2.4 % with a high mortality of 96-100%. Fluconazole is the treatment of choice for fungal infections; however invasive candidiasis and aspergillosis are relatively less susceptible to fluconazole. For many years, amphotericin B has served as the mainstay for the treatment of invasive fungal infections. However, infection related toxicity, nephrotoxicity and electrolyte disturbances have limited its use; moreover, the efficacy of amphotericin B is relatively limited with a mortality rate in treated patient that exceed 80%. Newer antifungal drugs, such as caspofungin and voriconazole, have been introduced recently in the treatment of invasive fungal infections and aspergillosis: these drugs have demonstrated to be as effective as amphotericin B in the treatment of invasive fungal infections with less drug-related side effects.

Methods: From January 2002 to December 2007, 247 kidney transplants have been performed at Organ Transplant Unit of University Hospital of Catania. Among these, 17 recipients developed an invasive fungal infection (13 an oesophageal infection and 4 an urinary tract infection), while 4 patients developed an invasive aspergillosis. The patients with invasive fungal infection have been treated as caspofungin, administered once-daily at a loading dose of 70 mg followed by 50 mg daily for the median of 87 days. We observe complete relief of symptoms in all patients. Caspofungin was well tolerated, with no signs of drug-related nephrotoxicity or hematotoxicity. Four patients with invasive aspergillosis were treated with voriconazole, starting with a dose of 200 mg b.i.d and was administered for a period of 60 days. All patients experienced a complete relief of symptoms without occurrence of aspergillosis.

Conclusion: Newer antifungal drugs, such as voriconazole and caspofungin are potent and well-tolerated antifungal therapies that are extremely efficacious in the treatment of invasive and aspergillosis and in the treatment of kidney transplant recipients. A careful monitoring of immunosuppressive drugs should be considered to avoid nephrotoxicity.

What happens with the large DNA when it meets intercalating drugs?

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Background: DNA intercalating drugs locally distort a regular DNA conformation. Usually any local changes of natural parameters cause some biological impact e.g. strange hindrance and change of binding affinities for DNA-associated enzymes. In case we know the molecular target, we are able to estimate optimal but efficient dosage of drug to eliminate many side effects. DNA is a huge macromolecule containing many potential places for intercalator binding. The sum of local changes causes a great global change in chromosome DNA. DNA intercalations might be a reason for chromosome reagangement and reassembling, causing inappropriate gene switching on and off.

Methods: Principal method is the temperature-gradient gel electrophoresis. The complementary methods AFM, UV-VIS spectroscopy and for cell cytotoxicity MTT test have been used. All obtained in vivo and in vitro results were extrapolated to chromosomal DNA.

Results: The table summarizes results obtained for selected intercalators, the reversibility of intercalator (ability to dissociate from DNA), where Cinf represents the concentration of drug measured by TEGE in 50 mM Tris- HCl (pH 7.8) extraplated to chromosome DNA. DNA intercalations might be a reason for chromosome reagangement and reassembling, causing inappropriate gene switching on and off.

Drug | Reversibility | Cinf (M) | C50 (M) | Ratio C50/Cinf |
--- | --- | --- | --- | --- |
Actinomycin D | 0.0-0.2 | 0.001 - 2.0 | 0.5 - 1000 |
Daunorubicin | + | 1.3 - 1.0 | 0.02 - 3.0 | 0.33 - 65 |
Ellipticine | + | 2.5 - 1.2 | 0.3 - 4.0 | 0.3 - 83 |
Quinacrine | + | 2.7 - 1.8 | 1.6 - 2.2 | 1.2 - 1.7 |
Quinine | + | 225 - 180 | 40 | 5.5 |
Quercetin | + | 230 - 150 | 20 - 50 | 4.6 - 11 |
Chloroquine | + | 20 - 22 | 17 - 33 | 0.6 - 1.2 |

Conclusion: For drugs where Cinf are ranked at 20-230 is not considered to be a standard cytotoxic drug, most likely because the probability that the intercalator will attain cytotoxic concentrations, eliminating any non-B structure from the DNA inside the cell. Therefore, only the cases where t=1.3 and Cinf <10 mM could play a significant biological role in forming alternative non-B structures. When the ratios are lower, cytotoxic processes such as the direct inhibition of one or more of the metabolic pathways.

Desipramine and Panic: Complex Approaches to Understand Complex Interactions in Psychopharmacology

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Background: Clinical studies have shown that some antidepressants may be more efficient than benzodiazepines to alleviate anxiety associated with panic disorder. Nevertheless, operant and conditioning procedures developed in rats so far do not seem particularly able to model human anxiety to anticonflict antidepressants. Researchers do not usually statistically subtract the effect of confounding factors of the variables of interest.

Methods: Undomesticated rats, which showed a generalized activation of cerebral and behavioral systems, were used. Operant and conditioning procedures developed in rats so far do not seem particularly able to model human anxiety to anticonflict antidepressants. Researchers do not usually statistically subtract the effect of confounding factors of the variables of interest.

Results: In the Geller-Shefter paradigm (under a cross over design) and the open field drink test (under an all-inclusive behavioral design) represented, respectively a decrease in the behavior of rats in the complex operant performance. The Diet x Drug interaction was independent of the effects of treatments on milk consumption, reactivity to the electric foot shock, unconditioned responding, weight and decision-making. In the Open Field Drink Test, the four dependent variables selected by factor analysis indicated also a significant Diet x Drug interaction in the two-way Manova. This interaction was independent of the effects of treatments on weight gain and was expressed, on deprived rats, as a decrease in the all selected open field-behaviors except for the time of drinking with respect to the control rats, which displayed, in general, a decrease in all the behaviors except for the frequency of grooming.

Conclusion: The Diet x Drug interaction was interpreted as a selective anticonflict effect of desipramine on subjects predisposed to develop panic-like expressions. Complex approaches allow more complete inferences than those which contemplate only one target behavior.
Study of Tenoxicam on Various Drug Delivery Systems

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Background: Tenoxicam is an antiinflammatory and analgesic agent which absorbs completely from the G.I. tract by oral route. It undergoes first pass metabolism and causes ulceration. Hence through the buccal and colon specific delivery system the drug circumvents the G.I tract and avoids the first pass metabolism and also the side effects frequently associated with oral administration like ulceration. Aims: (1) To evaluate the natural flux seed polymer by the buccal and colon specific delivery for tenoxicam. (2) To study the effect of the polymer for the bioavailability of the drug.

Method: The drug tenoxicam in the form of tablets containing various concentration of flux seed polymer (20, 30 and 40mg) were evaluated using invitro and invitro methods. The mucoadhesion of the tablets were evaluated using porcine buccal mucosa. The tablets had been subjected to invitro drug release experiments at a pH 6.8 phosphate buffer. The invitro study had been performed with 16 healthy human volunteers. In colonic delivery tablets of tenoxicam were prepared by compression coating with 400, 450 and 500 mg flux seed polymer. The formulated tablets were subjected to invitro drug release studies in simulated colonic fluids (4% w/v of rat cecal contents). The invitro study had been performed in 6 healthy human volunteers.

Results: The cumulative percentage release of tenoxicam at pH 6.8 phosphate buffer were found to be 98.20±0.08, 91.01±0.09, 84.39±0.72, 78.45±0.08. The bioavailability (AUC0-t*) of buccal and oral tablets was found to be 222±0, 228, 3221±0. The invitro drug release of colon specific tablets at pH 6.8 phosphate buffer containing 4% w/v rat cecal contents showed that the cumulative percentage release after 26 h were 52.16±0.06, 64.10±0.08 and 98.05±0.19. The invitro studies conducted in six healthy volunteers revealed that the drug release was initiated only after 5h (ie) transit time of small intestine and the bioavailability of the drug (AUC0-t*) was found to be 2014±0, 2890±0 and 2920±0.215.

Conclusion: (1) Buccal formulation of tenoxicam containing 40 mg flux seed polymer gives high bioavailability and also had significant mucoadhesive property for clinical application. (2) The colon specific formulation containing 400 mg flux seed polymer proved to have potential carrier for drug delivery into the colon for tenoxicam.

Anticancer Effects of Statins

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Background: Despite supporting theoretical reasons as well as experimental evidence, there is still clinical controversy whether statins (inhibitors of HMG-CoA reductase) can prevent from cancer diseases. These contradictory views are based on large epidemiological studies and their meta-analyses. Available data, however, have been focused primarily on cardiovascular outcomes; and secondly, based on large epidemiological studies and their meta-analyses. Available data, however, have been focused primarily on cardiovascular outcomes; and secondly, evidence, there is still clinical controversy whether statins (inhibitors of HMG-CoA reductase) have the possible anticancer action of statins have been studied as a group effect. Nevertheless, statins represent a heterogenous group of compounds, which differ in their physical-chemical properties, as well as different pharmacokinetic and pharmacodynamic properties.

Methods: Both experimental and clinical data available from Pubmed, as well as own results on the effect of statins on experimental pancreatic cancer, have been used for analysis of the chemopreventive effects of individual statins.

Results: Large differences in antiproliferative effects among agents used for clinical purposes have been described in available literature. In our experimental study on pancreatic cancer the least efficient statins were pravastatin and atorvastatin, whereas rosuvastatin (despite its low lipidophilicity) and cerivastatin were the most effective. These data may account for inhibitory effects on cellular growth/progression of the cancer cells, and the least effective statins mainly reduce cholesterol synthesis, whereas the most effective ones act directly on the cell cycle. The least effective statins, such as pravastatin and atorvastatin, are mainly used in pharmaceutical formulations.
Adenosine-Based Therapies for Hearing Loss

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Background: Oxidative stress is the key element in the pathogenesis of many forms of cochlear injury, for example from noise exposure, cytotoxic drugs and aging. Hearing loss from noise exposure (NIHL) is a leading occupational disease, with more than 10% of the population at risk world-wide. This study investigates adenosine-based experimental strategies to stem NIHL. Here we present the first viable pharmacological intervention that can ameliorate noise-induced cochlear injury in the post-exposure period.

Methods: Wistar rats were exposed to narrow-band noise (8-12 kHz, 110 dB SPL, 24 hours) sufficient to induce permanent hearing loss. Gene expression levels of adenosine receptors in the noise-exposed cochlea were studied using quantitative RT-PCR. Selective adenosine A1 receptor agonist Adenosine Amine Congener (ADAC) lacking cardiovascular side effects was administered intraperitoneally (100 µg/kg/day) at time intervals after noise exposure. Hearing thresholds were assessed using auditory brainstem responses (ABRs) and the hair cell loss was evaluated by quantitative histology. Free radical damage induced by reactive oxygen and reactive nitrogen species was assessed using 4-hydroxy-2-nitro-5-nitrosoyl nitrotyrosine markers respectively.

Results: Adenosine A1 receptors were up-regulated during sustained noise exposure suggesting their role in cochlear response to noise stress. The treatment with ADAC after noise exposure led to a substantial recovery of hearing thresholds at all frequency tested (4-24kHz). Earlier treatment with ADAC 16 hours after noise exposure provided greater recovery than late treatment starting at 24 hours post-noise. The results were upheld by increased survival of sensory hair cells and reduced nitrotyrosine immuno-reactivity in ADAC-treated cochlea. The activation of A1 adenosine receptors thus ameliorated damage to the sensorineural tissues in the cochlea, leading to improved recovery of auditory thresholds.

Conclusions: This study strongly suggests that ADAC could be a valuable treatment for noise-induced cochlear injury in instances of both acute and sustained exposure to noise and chronic or intermittent exposures of longer duration. ADAC may also become a drug of choice for other inner ear pathologies based on oxidative stress.

Authors’ disclosure statement:
All studies were approved by the University of Auckland Animal Ethics Committee. Supported by the RNID (UK), Deafness Research Foundation (NZ), Auckland Medical Research Foundation, and Health Research Council (NZ).

Budesonide - Breath-giving innovation for asthma patients

VOLOVITZ B

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Background: Asthma affects an estimated 300 million people worldwide, causing absenteeism from school and work, disability, and even death. Asthma was initially considered a disease of the smooth airway muscles, and treatment consisted of bronchodilators targeted at reducing the muscle constriction in order to ease the asthma. Its use has revolutionized asthma therapy and provided patients with a better quality of life.

The Development of Influenza Virus Sialidase Inhibitors as Anti-Influenza Drugs

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The important, and sometimes essential, roles of carbohydrates and the proteins that recognise them in biological processes are diverse [1]. When these molecules are associated with a disease a potential drug discovery opportunity is presented. For example, a range of clinically significant pathogens, including viruses, parasites and bacteria utilise carbohydrates and their associated proteins to invade their host, facilitate their lifecycle and as a consequence produce disease [2]. Viruses such as influenza virus, rotavirus, and dengue virus all have essential carbohydrate recognition processes in their replicative cycles that present possible drug discovery targets [2,3].

We have had a long-term interest in influenza virus and the discovery of novel influenza virus sialidase inhibitors that has now provided a novel class of anti-influenza drugs [4-6]. Some of our most recent work and advances towards the development of drugs to treat or prevent influenza, including pandemic influenza, will be presented.

multiresistant and hyper-virulent microbes have become a physicians’ nightmare in hospitals and in the community [USA300] [1]. These aggressive ‘superbugs’ demand ‘superdrugs’ addressing novel therapeutic approaches without cross-resistance to antibiotics in clinic. Novel natural products have been the most important source for new antibiotic drug classes [2]. Even today, natural antibiotic lead structures have not lost their value as guides for novel targets and future therapy (Reversed Genomics). Most natural products cannot be used in the clinics. However, medicinal chemistry provides the tools to transform natural products into drugs with improved pharmacokinetic and toxicological properties.

VON NUSSEBAUM, Germany

Multi-resistant and hyper-virulent microbes have become a physicians’ nightmare in hospitals and in the community [USA300] [1]. These aggressive ‘superbugs’ demand ‘superdrugs’ addressing novel therapeutic approaches without cross-resistance to antibiotics in clinic. Novel natural products have been the most important source for new antibiotic drug classes [2]. Even today, natural antibiotic lead structures have not lost their value as guides for novel targets and future therapy (Reversed Genomics). Most natural products cannot be used in the clinics. However, medicinal chemistry provides the tools to transform natural products into drugs with improved pharmacokinetic and toxicological properties.

We have investigated the medicinal chemistry of natural depsipeptide antibiotics such as the katanosines [3] or enepoptins as valuable lead structures on our search for new antibacterial therapies [4]. By their modular structure, cyclic peptides [5] are particularly well suited for systematic chemical modifications by means of semisynthesis and de novo synthesis. Synthetic strategies, structure–activity-relationships, in vitro potency and in vivo efficacy will be discussed.


Fentanyl Analogues: Structure-Activity-Relationship (SAR) Study


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Background: Fentanyl is the prototype of the 4-anilidopiperidine class of synthetic opioid analogues. This study aimed to examine antinociceptive activity of newly synthesized fentanyl analogues substituted at the position 3, or at the position 4 of the piperidine ring, and to establish structure-activity-relationship (SAR).

Methods: Synthesized fentanyl analogues substituted at the position 3, or at the position 4 of the piperidine ring, and to establish structure-activity-relationship (SAR).

Conclusions: The potency and the duration of action of these novel fentanyl analogues are interesting from the aspect of SAR studies, and some of them have the piperidine ring, and to establish structure-activity-relationship (SAR).

Synthesis of a Novel Subunit Vaccine against HIV-1: Native Envelope Proteins in Lipid Bilayer of Inactivated Virions Devoid of p24, RT and Viral Nucleic Acids

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Background: HIV vaccine development remains a continuing challenge. It is our objective to prepare inactivated HIV-1 isolates devoid of p24, RT, and viral RNA, but retaining the dendriticcellary structure of their native envelope proteins. A pool of such isolates derived from human blood can safely elicit protective antibodies against HIV-1.

Methods: Plasma from blood donors with acute HIV infection (PHIV: NAT+ but antibody-) was used for virus isolation. PHIV isolates co-cultured with PMA-stimulated human PBMC from transfusable blood were pooled and reacted with Benzonase (BZ) to hydrolyze free cellular viral DNA/RNA and filtered through a 300 kD membrane. Beta-cyclodextrin (BCD) was used to extract membrane cholesterol and permeabilize the purified virions, allowing BZ to hydrolyze virus-associated DNA/RNA. The quaternary structure of HIV-envelope proteins (gp41 and gp120) was restored by reincubating the virions with cholesterol.

Results: The PHIV isolates co-cultured with single donors’ PBMC yielded p24 widely ranging between 2.6-175 ng, whereas PHIV pooled from 3-4 unselected donors uniformly yielded 174-177 ng/ml cells. Magnetic beads coated with anti-CD45 removed cellular microvesicles from culture supernatants, yielding purified virions (0.6 pg/mL p24), which contained virus RNA –2X10^6 copies/mL (CpmL). After reaction with 300 or 500 mM BCD, virions were consistently negative in PMAC co-cultures. Results tabulated below show residual p24 and HIV-1 RNA CpmL after each successive step of viral inactivation.

Thus, viral inactivation with BCD led to loss of <10^6 HIV-1 RNA CpmL ampltitude by RT-PCR; this was further reduced to 0.8X10^4 by additional reaction with BZ to hydrolyze virus-associated RNA. Since HIV-1 SF2 vaccine stock contained ~10^6 TCID50 and 6.4X10^6 HIV RNA CpmL, the minimal chimpanzee infectious dose (CID50) of 1X10^2.3 is equivalent to ~5.4X10^3 CpmL. Thus, combined treatment with 0.3 mM BCD and BZ would provide a product with safety margin that is ~2X10^3 below the minimal in vivo infectivity in chimpanzees. The foregoing results indicate the safety and efficacy of PHIV inactivation, which eliminated p24 and HIV-1 RNA but retained ~65-95% of the gp120.

Conclusions: Our preliminary results provide an impetus for pragmatic refinements and a national basis for seeking FDA-ind and IRB approvals for a pilot clinical trial of the candidate vaccine for immunotherapy in individuals whose HIV infection is controlled by HAART. Ultimately, broadly neutralizing antibody responses in uninfected-risk individuals would permit serological distinction between infection and protective immunization.
Proteomic analysis of human breast cancer cells derived from metastatic versus non-metastatic tumors

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Background: Breast carcinomas are a heterogeneous group of tumors diverse in behavior, outcome and response to therapy. Identifications of proteins that reflect the tumor biology can improve the diagnosis, prediction, treatment selection and targeting of therapy.

Methods: Primary cultures of epithelial cells from malignant breast tumors were studied by two-dimensional electrophoresis (2DE). Differentially expressed protein spots were identified by mass spectrometry. The samples were obtained from women who underwent partial breast resection or radical mastectomy for breast cancer at the General Faculty Hospital in Prague. The patients were treated according to stage-adjusted therapeutic standards. We estimated the clinical outcome of the patients. The 23 patients with follow-up at least three years were chosen for further analysis. The patients were divided in two groups: distant metastases-free after three years and patients with proven distant metastases. 2-DE gels in pH range 4-7 were prepared. Spot densities in 2-DE protein maps were subjected to statistical analyses and data-mining analysis. Proteins in selected spots were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: Three protein spots were significantly altered between the metastatic and nonmetastatic groups. The correlations were proven at the 0.05 significance level. Nucleophosmin was increased in the group with metastases. The levels of 2,3-trans-enoyl-CoA isomerase and glutathione peroxidase 1 were decreased.
Absorption, Kinetics, Metabolism and Disposition of the Renin Inhibitor Drug Aliskiren

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Background: Aliskiren is the first in a new class of orally active, non-peptide direct renin inhibitors developed for the treatment of hypertension. Over decades, efforts to develop high affinity inhibitors failed, but the recently developed aliskiren was highly potent, due to its oral bioavailability. Aliskiren was designed based on crystallographic analysis and molecular modelling. After oral bioavailability had been found in animals, clinical development was initiated and clinical efficacy and safety could be shown. Aliskiren was found to be a highly potent inhibitor of renin in humans. The hypothesis that inhibition of renin results in low plasma renin activity (PRA) and low aldosterone production. Measurements: We investigated the pharmacokinetics of aliskiren in human volunteers. Methods: After oral administration of aliskiren, the plasma and urinary concentrations of aliskiren and its active metabolites were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Results: Mean plasma exposure after oral administration of 150 mg aliskiren is shown in the figure. Conclusions: Aliskiren is a highly effective inhibitor of renin and has a similar therapeutic profile to spironolactone. However, Aliskiren is associated with a risk of hyperkalemia, which may limit its use in patients with renal impairment.

Progestosterone and its Metabolites Have Functional Effects on Processes other than Pregnancy, such as those involving Stress, Affect, and Cognition

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Background: Although progestosterone (P) and its products, such as 5α-pregnane-3α,20α-dione (3α,5α-THP; allopregnanolone), are typically thought of in their role in maintaining pregnancy, P and 3α,5α-THP can have wide ranges of other functional processes. There are data from clinical studies and animal models that 3α,5α-THP can enhance various other functional processes. However, 3α,5α-THP has actions in the midbrain ventral tegmental area (VTA) to modulate the intensity and duration of lordosis and actions in the hippocampus to reduce anxiety-like behavior. Furthermore, 3α,5α-THP increases in midbrain as well as the hippocampus in response to reproductive, as well as stressful/moosum, stimuli. Of interest are the effects of progestins for other hippocampally-mediated processes, such as cognition. Methods: We have conducted studies investigating the effects of progestins in the hippocampus in young and aged rodents to test the hypothesis that 3α,5α-THP can have mnemonic effects. In each, the main endogenous source of P was removed (the ovaries). P or 3α,5α-THP or placebo vehicle was administered to experimental subjects, and cognitive performance was assessed. Results: We found that young ovariectomized rats or mice, as well as aged mice (which have naturally low progesterin levels) administered treatments that enhance 3α,5α-THP (P or 3α,5α-THP), but not those that do not enhance 3α,5α-THP (placebo vehicle, medroxyprogesterone acetate) have enhanced cognitive performance. In a transgenic mouse model of Alzheimer’s Disease, there were concomitant deficits in hippocampus-mediated processes and 3α,5α-THP elicits following long-term P administration, compared to that observed in wildtype mice. Conclusions: These data suggest that progestins can have functional effects beyond their modulation of reproduction and affective behavior to influence mnemonic processes through actions in the hippocampus.
Bioartificial Human Tissues as Model Systems for Pharmaceutical Target Screening and Drug Development

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Background: In order to translate the findings from basic research into clinical applications, cell-based models need to recapitulate both the three-dimensional (3D) organization and multicellular complexity of an organ. Tissue engineering provides new perspectives for basic and applied research by offering applications, cell-based models need to recapitulate both the three-dimensional and functional evidence of dedifferentiation. Biochemical testing revealed stable metabolization. Using lymphangioma-tumor tissue, a new tumor cell type could be identified. The bioartificial liver tissue was viable for 3 weeks without histological differentiation was controlled by histology, immunhistochemistry, cytokine release, metabolite release, and tissue specific microarrays. In preliminary studies, the metabolic activity of bioartificial human liver-like tissue was characteristic.

Results: Co-culture conditions for various cell types were successfully developed to generate bioartificial human skin, liver, airway and eventually intestinal tissue. These tissues could be applied for applied research: i) drug diffusion, ii) drug resorption, iii) tumor penetration, iv) circulatory distribution and v) drug metabolism. Using lymphangioma-tumor tissue, a new tumor cell type could be identified. The bioartificial liver tissue was viable for 3 weeks without histological and functional evidence of dedifferentiation. Biochemical testing revealed stable metabolic activity of the tissue culture. The bioartificial airway model showed coordinated ciliary activity for more than two weeks.

Conclusions: Bioartificial human tissues represent versatile model systems for pharmaceutical target screening and drug development, complying with the European Union’s REACH regulations. However, their effective potential in expediting drug development and testing is subject of ongoing research.

Chronic Levonorgestrel Treatment in a Macaque Species (Macaca sylvanus): effects on sex steroids and secondary sex characteristics

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Background: Administered levonorgestrel enables contraception under normal cyclicity of the ovaries and does not suppress the development of cyclic perineal swellings. This study investigated the influence of levonorgestrel on gonadal steroid excretion rates and the expression of perinatal swell size.

Methods: Two groups of Barbary macaque females were observed: Twenty-four implanted individuals under semifree conditions and five non-implanted individuals under caged conditions. Eight of the implanted females had large expressions of progesterone concentrations in the feces compared to individuals with reduced progesterone titers can be judged as an endocrine indicator for the expression rate of perineal swelling size.

Antimicrobial Resistance in South Africa: ‘Crawling for a Magic Bullet’

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Background: Antibiotic resistance (AR) is a problem in South Africa owing to their wide spread use. Several studies have shown that resistance is of concern in hospitals due to nosocomial infections, but community acquired infections have also exhibited change in the resistance pattern. Also, antibiotic resistance in public health facilities appears to be different from that in private institution. Unfortunately, the information is reported in different articles. Here, we undertake a review and analysis of these reports with a hope that it will improve accessibility and utility in the fight against AR.

Methods: A search for relevant abstracts during 1966 to 2007 was done on Medline database using search terms that included: South Africa, antibiotic, resistance and prevalence. The full length articles of the relevant abstracts were evaluated for inclusion in the review. Other relevant articles were obtained from local journals. Results were reported as average prevalence (%) of AR with time or to multiple antibiotics.

Results: Thirty-four abstracts were recovered and, of these, 9 articles qualified to be used in this review. In survey of 7 academic hospitals (2001-2004), the prevalence of MRSA was 46.4%, while in a 6 month study of 12 private laboratories, it was 36%. The prevalence of Penicillin-resistant pneumococci (PRP) was 45% in 1997, having risen from 31.3% in 1996. In another report, the prevalence of PRP in community acquired Lower Respiratory Tract Infections increased from 29.4% in 1996 to 35.6% in 1997, while the prevalence of β-lactamase producing H. influenzae in one academic hospital rose from 33% in 2001 to 40% in 2003. Regarding multiple AR the prevalence was gentamycin 45.6% and ciprofloxacin 11.3%, while that of Ps. aeruginosa it was, moxopenem 42%, imipenem 45%, cefepime 53% and ciprofloxacin 46%.

Conclusions: 1) The prevalence of AR in South Africa is very high (> 20%), on an upward trend, and multiple AR on the increase. 2) There was increased use of the newer antibiotics such as moxofloxacin, levofloxacin, telithromycin for community acquired infections, and the menoperoxidase and vancomycin for nosocomial infections, and this has lead to emerging of resistant strains to these drugs. 3) There is a need for new and more effective antibiotics before we run out of options. In effect, South Africa is crawling for a Magic Bullet.
Therapeutic protein engineering via the incorporation of non natural amino acids

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Incorporation of unnatural amino acids into recombinant proteins represents a powerful tool for protein engineering and protein therapeutic development. The presence of the unnatural amino acid in recombinant protein enables site-specific modification of protein at these unnatural amino acids. For example, site-specific pegylation of protein extends the in vivo half-life of therapeutic protein, while site-specific conjugation of antibody to toxin has long been regarded as ‘guided missile’ in treating cancer patients. In this study, we have engineered human interferon-beta protein with two unnatural amino acids, L-acetyloxy-L-ornithine (AHA) and L-homopropargylglycine (HPG). We report the effects of the penultimate residue (the residue after the initiator Met) on the processing of AHA and HPG, at the N-terminus of recombinant human interferon-beta in E. coli. We have identified specific amino acids at the penultimate position that can be used to efficiently retain or remove N-terminal AHA or HPG. Retention of N-terminal AHA or HPG can be achieved by choosing amino acids with large side-chains (such as Gin, Glu and His) at the penultimate position, while Ala can be selected for the removal of N-terminal AHA or HPG. We also report site-specific pegylation at AHA site and characterization of pegylated IFN-beta protein.

On the Delivery of Carmustine, Paclitaxel, and Etanidazole to Brain Tumors: An integrated study on the novel fabrication methods of pharmaceutical particles and 3-D computer simulations for chemotherapy and radiotherapy applications

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Conventional treatment of brain tumor involves excising the main part of the tumor in conjunction with post-surgery treatments such as chemotherapy, radiotherapy, or a combination of both. One of the difficulties associated with such treatment is that the tumor mass cannot be completely removed without the risk of damaging neighboring healthy tissue. In addition, the tumor mass remains capable of rapid multiplication and encroachment into nearby healthy tissue. In traditional chemotherapy, anti-cancer agents are delivered potentially to the malignant site via systemic administration in vivo. However, the presence of high drug resistance in brain tumors limits the therapeutic effects. Consideration of the possibility of using new delivery platforms to control the delivery of chemotherapeutic drugs to the tumor site and to enhance the efficacy of chemotherapy and radiotherapy by local drug delivery is attractive.

The second part of this paper further reports the development of three-dimensional computer simulation models to study the optimal location of polymer implantation. Two types of drug deliveries, namely, systemic administration and controlled chemotherapy and radiotherapy applications. The study yields information on the efficacy of various delivery methods, and effectiveness of the chemotherapy combined with local treatment by fibronectin. This is better than the routine chemotherapy for the MDR-cavitary pulmonary TB.

Nucleotide Excision Repair (NER) in cisplatin-induced cellular responses

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Cisplatin is an important anticancer drug that has been used in the treatment of many types of cancer. Cisplatin binds to the DNA template to form both intra- and inter-strand crosslinks (ICL). Recognition of this ICL damage can eventually lead to apoptosis cell death, which is an important mechanism for cisplatin in its anticancer action. Nucleotide excision repair (NER) plays an important role in removing DNA damage generated by cisplatin. The results obtained from our studies reveal that the DNA damage recognition signal initiated by XPC protein, an important DNA damage recognition protein for the NER, is essential for cisplatin-induced cellular responses such as cell cycle arrest and apoptosis. The DNA damage recognition signal of XPC protein is required for cisplatin-induced p53 phosphorylation, while the XPC-TFIIH complex formation plays a key role in the p53 phosphorylation. The phosphorylated p53 protein further regulates transcriptions of a series of downstream target genes to result in cell cycle arrest and/or apoptosis. Furthermore, the process of cisplatin DNA damage by NER also causes activation of the ATM protein, which then induces transcription of an important anti-apoptotic bcl-2 gene through NF-κB, resulting in increased accumulation of Bcl-2(L) and subsequent cell survival under cisplatin treatment. Therefore, the NER possesses two very distinct roles in the cisplatin-induced cellular responses: (1) the DNA damage recognition signal initiated by the XPC protein is essential for cisplatin-induced cell cycle arrest and apoptosis; and (2) the NER process of cisplatin DNA damage is required for ATM activation and cell survival under cisplatin treatment. Therapeutic manipulations that enhance the DNA damage recognition-induced cell cycle arrest and apoptosis and repress the NER process will significantly improve the effectiveness of cisplatin in anticancer treatment and reduce tumor cell resistance to cisplatin.
Assessing the Metabolic Liabilities of Aromatic Amines using In Vitro Metabolism and Mass Spectral Techniques

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Background: The purpose of this study was to establish a combination of in vitro metabolism and mass spectral techniques that could potentially be used to assess metabolic liabilities of aromatic amines in mutagenicity. An attempt was made to correlate the Ames results with formation of the deoxyguanosine adducts.

Methods: 1) Aminofluorene (2AF) was used as a model compound that was bioactivated in the presence of rat (aroclor induced) liver S9 fortified with NADPH and N-acetyl CoA. Deoxy-guanosine (dG) was used as a nucleophilic trapping agent. A covalent adduct of 2AF-dG, the formation of which depended on the presence of CYP1A2 and NAT2 in the incubations, was characterized by LC/MS. By employing the technique 11 model compounds, their mutagenicity have been demonstrated. 2) Quantitative LC/MS analysis of the deoxy-guanosine adducts formed with the 11 target compounds demonstrated that the correlations were 0.85 and 0.54 in ranking the reactivity of the model compounds by the peak area of dG of LC/MS vs by revertants/nmol of the trapped adduct. These preliminary results suggest that this technique could potentially be applied to guide synthetic efforts in mitigating bioactivation of such compounds.

Effect of Genetic Polymorphisms in ABCG2 (BCRP) on Inhibition and Potential Drug Resistance

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ABCG2 (Breast cancer resistance protein, BCRP, MXR) is an adenosine triphosphate-binding cassette transporter that effluxes drugs and metabolites out of cells such as anticancer compounds. SNPs in the human ABCG2 gene have been associated with altered drug response. To determine the role of genetic polymorphisms in ABCG2 on sensitivity to inhibition and drug resistance, we investigated the potential effect of ABCG2 polymorphisms on drug transport.

Methods: SNPs were selected from previously described polymorphisms in the ABCG2 gene and the sensitivity of drugs to these SNPs was evaluated. The expression of ABCG2 in various cell lines was determined using RT-PCR. Confocal microscopy demonstrated that all the ABCG2 variants were localized to the cell membrane. A fluorescent inhibition assay was developed and was used to measure IC50 against 13 compounds for ABCG2 WT and variants. The results showed that SNPs can have different IC50 against certain inhibitors, indicating that SNPs can play a potential role in drug resistance, and when using a combination of agents that is an ABCG2 substrate/inhibitor, the impact of genetic variation should be considered.

Abstracts Page A-349
New multifunctional pharmacophores and biocompatible nanocomposites for targeting drug delivery and cancer diagnosis

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It is well known that the failure of chemotherapy to the malignant tumor is usually induced by multidrug resistance (MDR) and the development of effective anti-MDR agents for efficient drug delivery plays an important role in the tumor therapy. Multidrug resistance (MDR) is the ability of disease-causing organisms to withstand a wide-variety of structurally and functionally distinct drugs or chemicals. MDR involves the expression of membrane proteins which mediate the active ejection of drugs from the cell and it is a major limiting factor to the chemotherapy of cancer. Because of the importance of MDR in clinical oncology, an extensive search for the new MDR reversal agents is still an unceasing challenge. Many compounds known to have other pharmacological sites of action initially were used to reverse MDR in cancer cells grown in culture and several underwent pilot clinical trials. A large number of small molecules capable of modulating P-gp mediated MDR have been described in the literature. Among the potentially pharmaceutical functional molecules, the remarkable chemical and thermal stability and hydrophobic character allow the carbonate to be used as a promising pharmacophore in biologically active molecule anti-drug resistance and anti-cancer application. Our recent studies indicate that when co-administered with a cytotoxic agent, these nontoxic modulators enhance net accumulation of relevant cytotoxic drugs within the tumor cells. In recent years, nanomaterials, which show unique physical and chemical properties, have attracted much attention in various fields and have been widely applied in biological and biomedical engineering. In this report we will present some of our recent research progress in combining the design of multifunctional biocompatible promising pharmacophore and nanomaterials with targeted and efficient drug delivery system. These studies demonstrate that with the combination of multifunctional pharmacophore and nano-interface, we can realize the synergistic effect on the efficient cytotoxicity suppression in drug-sensitive and drug-resistant cancer cells and enhance intracellular drug accumulation of anticancer drug into target cancer cells. Meanwhile, our observations also indicate that due to the convenient surface functionalization, the biocompatible nanomaterials can play versatile roles for fabrication of high sensitive probes and biosensors in cancer diagnosis, which show great promise in the design of point-of-care-testing devices in the future.

Acknowledgements: This work has been supported by National Natural Science Foundation of China (20713023, 20675014) and Ministry of Science & Technology of China (2007AA022007).

From Bark to Bullet: A Personal History of the Discovery and Development of Taxol

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Taxol is a secondary metabolite obtained from the wood bark of Taxus brevifolia, found in the Pacific Northwest coastal region of the United States. It was isolated by the bioassay-guided fractionation of the crude plant material. The structure of Taxol was established by single crystal x-ray analysis. Taxol has a unique mechanism of antitumor activity. It inhibits cancer cell growth via stabilization of microtubules. Currently, Taxol is approved for clinical use in the USA by the FDA for the treatment of refractory ovarian, breast, and non-small cell lung cancers and Kaposi’s Sarcoma. This presentation will describe the 30-year efforts which transformed this compound from an interesting plant secondary metabolite to a life saving chemotherapeutic agent.

Increased PGC-1 expression exhibits protective effects against age-related neurodegenerative and metabolic disease processes

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Background: It is generally accepted that mitochondrial function declines with age, as evidenced by the increased level of cellular oxidative stress due to increased mitochondrial production of reactive oxygen species (ROS). It is also widely accepted that age-related alterations of mitochondrial function constitute a major component of the aging process. The transcription co-activator peroxisome proliferator-activated receptor co-activator-1 (PGC-1) is known to be a powerful stimulator of mitochondrial biogenesis. We were therefore interested in determining if stimulation of mitochondrial biogenesis would lead to an improvement in mitochondrial function and thereby modulate the aging process.

Methods and Results: To this end a unique transgenic animal model has been developed in which the human PGC-1 gene has been inserted into the C57Bl6/J mouse genome. Ubiquitous 2 to 3 fold increases in the expression of human PGC-1 mRNA and protein was observed. These changes were accompanied by 2 to 4 fold increases in mRNA and protein of transcription factors and other proteins associated with mitochondrial biogenesis (mTFA, COX II, etc.). Unexpectedly, we found that the transgenic animals exhibited an improvement in the glucose tolerance response. Use of the euglycemic-hyperinsulenic clamp technique demonstrated that this is due to increased skeletal muscle insulin sensitivity. Another unexpected finding was that cross-breeding the PGC-1 animals with the G93A mouse model of amyotrophic lateral sclerosis (ALS) exhibited a rescuing effect as evidenced by the extension of life span and marked improvement in RotoRod performance. This finding is consistent with the reports that increased expression of PGC-1 exhibits a rescuing effect in a murine model of Huntington’s disease.

Conclusions: Alterations of PGC-1 expression, or activity, may therefore provide a mechanism for altering the progress of a metabolic disease such as Type II diabetes mellitus as well as in neurodegenerative diseases such as ALS and Huntington’s disease. (Supported by NIH grant AG028294)
Targeted Therapy of Human Neuroblastoma Cells using Auger Electrons of Indium-111-labeled N-myc Antisense Oligonucleotide

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Background: Auger electrons may destroy nucleic acids in the close vicinity, and thus targeted therapeutic effect of Auger electrons of indium-111 (in-111)-phosphonate antisense oligonucleotides on human neuroblastoma cells has the overexpression of N-MYC was studied.

Methods: Human neuroblastoma cell SK-N-DZ (5 x 10^5) cells were treated for 20 hours with cationic reverse-phase vesicles encapsulating In-111-antisense oligonucleotide (AS) (average specific radioactive of vesicle of 20 MBq) nmol of oligonucleotides/nmol of total phospholipids) with average diameter of 250 nm. The expression of N-MYC and proliferation of the treated tumor cells were investigated as well as the existence of In-111-AS hybridized with intracellular N-myc mRNA. Tumor genicorticity of the treated tumor cells was examined in nude mice. As a control, non-radioabeled AS or In-111-sense oligonucleotides (S) were employed.

Results: The In-111-AS hybridized with intracellular N-myc mRNA were detected from the treated tumor cells at 12 and 24 hrs after initiation of the treatment. Reduction in the expression of N-MYC (average 27% to control) and inhibited cell proliferation (average 59% to control) were shown in cells at 48 hrs after treatment of In-111-AS. The N-MYC-suppressed cells however continued to produce tumor although a significant decrease in average weight of tumors was demonstrated in intraperitoneal cavity of nude mice. Neither of non-radioabeled AS nor In-111-S caused any effect in tumor cells.

Conclusions: Auger electrons of In-111 in the close vicinity of their target N-myc mRNA may cause reduction of cell proliferation rate of human neuroblastoma cells with the overexpression of N-MYC, and In-111-antisense oligonucleotides could potentiably be a tool for internal molecular radiotherapy at a level of mRNA of a tumor cell.

The Tissue Specific Choice of Anti-cancer Drugs on the Basis of Polymamine Level

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Background: The Growth Factor-Ras-ERK signaling pathway is frequently perturbed in human cancers, and is a target for the development of tumour therapies. The Ras-ERK signalling pathway targets various cellular effectors, including the Net (ErbB-3/AFAP-2/Erp) transcription factor, which is phosphorylated and activated by ERK, and is involved in wound healing, angiogenesis and tumour growth.

Methods: A cell based screen for small molecule inhibitors of Ras activation of Net transcriptional activity was used for a high throughput screen for small molecule inhibitors of the pathways. Selected molecules were tested for their activity, specificity and mechanisms of action using in-vitro and in-vivo molecular and cellular based approaches.

Results: We identified a novel pyrazole XRPP4X. XRPP4X inhibits FGF-2 induced Net phosphorylation by the Ras-ERK signaling upstream from Ras. It also binds to the colchicine-binding site of tubulin, depolymerizes microtubules, stimulates cell membrane blebbing and affects the morphology of the actin skeleton. Interestingly, XMR3-4A, which produces similar effects on the cytoskeleton, also inhibits FGF-2 Ras-Net signaling. This differs from other classes of agents that target microtubules which have either little effect (Vincristine), or no effect (Docetaxel and Taxotere), on the Ras-Net magic bullet site. XRPP4X has the potentially interesting property of connecting two important pathways involved in cell transformation. It is an interesting molecule for the development of cancer treatment.
When More is Not Necessarily Better: Interdisciplinary Inquiry into the Implications of U-Shaped Dose Responses for Personalizing Anticancer Interventions

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Background: The perception that is pervasive among the public is that, when it comes to cancer-fighting dietary supplements, more is better. Whether or not this concept is valid is especially relevant to health-conscious men and women, who are ironically at highest risk for the ill-effects of oversupplementation because they are already consuming high quality diets rich in vitamins and minerals. In 2001, the National Cancer Institute launched SELECT to evaluate whether daily supplementation with selenium (Se) or vitamin E prevents prostate cancer. But very little was known about what dose of Se might offer the most potent cancer-protective effects. We hypothesized that Se regulates the accumulation of genotoxic damage within the prostate and that the relationship is non-linear, i.e. more Se is not better.

Methods: We conducted a randomized feeding trial in which 69 elderly beagles (equivalent to 65 year-old men) received adequate or supranutritional Se intake for 7 months. We used the aging dog prostate to mimic the aging human prostate, enabling us to study the effects of Se on prostatic cells in an appropriate context.

Results: Se supplementation significantly decreased the accumulation of DNA damage in the prostate (alkaline Comet assay). When we examined the relationship between toenail Se level and prostatic DNA damage, we discovered an intriguing U-shaped dose response curve: more was not better. Further, we showed that the Se level that minimizes DNA damage in the aging dog prostate remarkably parallels the Se level that minimizes prostate cancer risk in 2 large human studies.

Conclusions: Now, more than ever, we need a new approach to cancer prevention — personalized cancer prevention (Waters et al, Nutrition and Cancer 2008; 60:1-6). Defining the U-shaped relationship between DNA damage and cancer-modulating nutrients addresses one of the major obstacles to developing personalized cancer-reducing interventions. It follows from this understanding that not all individuals will necessarily benefit from increasing their nutrient intake. Baseline nutrient status should be required for all individuals in prevention trials to avoid oversupplementation.

Early Results of Imatinib as the Active Agent in Control of Aggressive Fibromatosis. An Attempt of Metanalysis

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Background: Aggressive fibromatosis (AF) is a neoplasm arising from musculoaponeurotic structures with fibroproliferative characteristics. Most patients suffer from the abdominal tumors and other locations. Molecular pathobiology appears to be of clinical value especially when there is a possibility to use targeted therapy with imatinib. This drug can block several molecules to imply clinical benefits in patients whose advanced AF tumors are unresistant and progressed after completion of radiation, and systemic therapy.

Methods: We have described our clinical experience and have searched databases (PubMed, and Medline) to find publications focused on the imatinib therapy for advanced AF. Early results have shown the clinical activity of imatinib as the salvage therapy in patients with advanced AF.

Results: We found only a few publications focused on the role of imatinib as the salvage therapy in advanced AF. The total number of 1175 patients with advanced AF were given imatinib at the dose of 400 mg orally daily for more than 2 months. Median treatment time was 9 months. Median time to progression of AF on imatinib was 6.8 months. 13 patients were given 800 mg orally daily when progression of AF on 400 mg of imatinib was recorded. Among them in 7 patients SD (stabilization disease) was noted. There were following responses to imatinib assessed in 175 patients: 2 CR-1.1% (complete response), 26 PR-14.8% (partial response), 99 SD-56.5%. 127 patients (72.5%) experienced clinical benefits interpreted as disease control. 47 patients had toxicity profile of G1-3 (grade) (no G-4): asthenia-44.6%, nausea and vomiting-40.4%, diarrhoea-25.5%, oedema-25.5%, abdominal pain-12.7%, rash-12.7%. There were also attempts to characterize AF at the molecular level by expression of following: KIT-26 positive/44 patients, PDGF receptor alpha-24 positive/44 patients, PDGF receptor beta-31/44 patients, mutations within PDGF receptor alpha exon 12, and PDGF receptor beta-28/44 patients, within PDGF receptor beta exon 18-3/22 patients. Additionally, WNT was mutated in 84% (16 cases/19 patients) but no correlation between mutations and responses to imatinib was noted.

Conclusion: Imatinib is an active drug that is able to be used to control advanced AF remaining unresistant and after completion other therapeutic modalities as radiation, and systemic treatment with anti-inflammatory drugs, endocrine therapy, and chemotherapy.

Abstracts listed are alphabetically ordered of the presenting author.
Residue Networks and Drug Resistance in Hepatitis C Virus Antiviral Therapy

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Nürnberg, October 3-5, 2008

Background: Telaprevir (RX-950) and Boceprevir (SCH 503034) are hepatitis C virus (HCV) NS3-4A protease inhibitors with strong antiviral activity in phase I clinical trials and currently in phase 2 development. We revealed amino acid residues undergoing varying degrees of conformational flexibility and clonal analysis for both inhibitors. We apply in-silico approaches and the HCV replicon system to study the molecular mechanisms of drug resistance.

Methods: We evaluated experimental structures of NS3-4A, together with protein-ligand docking, rotamer analysis and molecular dynamics simulations. Furthermore, we introduce a novel approach using a 2D network formed by the non-covalent interactions between amino acids of the 3D protease structure to interpret the effect of important mutations on replication efficacy and resistance development. An HCV replicon assay was used for IC50 determination of mutant NS3-4A proteases.

Results: We investigate the conformational variability of the protease in alternative ligand-binding models and predict the binding mode of RX-950. We use the 2D network of non-covalent interactions to discover mechanisms of drug resistance in VX-950 and SCH 503034. Mutations at positions 36 and 54 are located spatially close to a hydrophobic cavity in the ligand-binding pocket. We show that the cyclopropyl group in VX-950 is oriented towards this hydrophobic cavity. We also describe the potential impact of mutations at V36 and T54 result in impaired interaction with the VX-950 cyclopropyl group, which explain the development of viral breakthrough variants in VX-950 but not in SCH 503034.

Conclusions: 1) T54 mutations are expected to interfere with the catalytic triad of the protease and thereby are assumed to affect the viral replication efficacy to a larger degree than V36 mutants. Molecular dynamics simulations of T54A/S mutants and rotamer analysis of V63A/GLM side-chains support our interpretations. 2) Mutations at V36 and T54 result in impaired interaction with the VX-950 cyclopropyl group, which explain the development of viral breakthrough variants in VX-950 but not in SCH 503034.
**Abstracts**

**Black:**

**The Anti-Tumor Action of the Hybrid Drug Nitric Oxide-Donating Aspirin**

Relies on the ‘Passive’ Linker and not on Nitric Oxide Nor on Aspirin: An Overview of an Interesting Twist

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**Background:** Hybrid drugs, by design, rely on the combined action of two pharmacologically active units in one molecule. The hybrid drug nitric oxide-donating aspirin (NO-ASA) consists of aspirin (ASA) and a nitrate group connected through a chemical linker. Its design was based on the assumption that NO, released from the nitrate group, would counterbalance the gastrointestinal side-effect of ASA. Benchmark derivatives are meta-NO-ASA and para-NO-ASA, both having a benzyl-type linker. Both meta-NO-ASA and para-NO-ASA proved to be active anti-tumor agents in preclinical models, generating excitement in the hybrid drug community. Our aim was to study the actual contribution of the nitrate and ASA hybrid components in the most active derivative, para-NO-ASA.

**Methods:** Structural analysis of NO-ASA led us to synthesize model compounds in which the nitrate- and/or ASA-moiety in para-NO-ASA were replaced by a ‘dummy’ chloro- and/or acetyl-group, respectively, but with retention of the benzyl-type linker. Organic and computational chemistry as well as spectroscopic analyses were used to study the chemical characterizations of NO-ASA and model compounds. Furthermore, we studied the mode of action of these compounds in colon cancer cells, including the effect on glutathione levels, caspase-3 activation, cyclin D1 expression, DNA fragmentation and cell death.

**Results:** All the synthesized model compounds, i.e. lacking ASA and/or the NO-donating group, retained or improved upon the in vitro anti-tumor activity of NO-ASA in colon cancer cells. They do so by exhibiting similar (bio)chemical effects as para-NO-ASA does. We show that this is a result of a unified mechanism involving formation of a cytotoxic quinone methide from the ‘passive’ benzyl linker through intramolecular hydride transfer and subsequent elimination of an intact nitrate ion. This quinone methide depletes intracellular glutathione by a selective chemical reaction.

**Conclusions:** Both NO and ASA, paradoxically, are not involved in the in vitro anti-tumor action of para-NO-ASA. Rather, a cytotoxic quinone methide formed from the ‘passive’ linker is the responsible agent.

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**ENR:**

**Transformation and Environmental Fate of the Veterinary Fluoroquinolone Enrofloxacin**

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**Background:** Fluoroquinolones (FQs) are synthetic antibiotics widely used to treat bacterial infections in humans and animals. Hence, drug residues enter the environment via urine and feces, i.e. in wastewater streams and animal wastes, respectively, the latter often being spread as fertilizer on agricultural fields and pastures. Due to very tight binding of FQs to feces and soils as well as a fluoro- aromatic structure element not yet found in natural products, concerns have been expressed on FQ biodegradability, long-term persistence, and potential selection of FQ-resistant zoonotic species in the environment. Metabolic transformation of enrofloxacin (ENR), a veterinary FQ, is limited to glucuronidation and partial oxidation of its amine substituent. In the only soil degradation study yet published (1997), 20.6% of the 14C label applied with [2-14C]enrofloxacin could be recovered as 14CO2 after 80 days, prompting our work on the fate of ENR in agricultural matrices.

**Methods and Results:** In samples of pre-rotted wheat straw, cattle dung pats, a manure hill, and an agricultural soil only about 0.5% of 14C was produced from [4-14C]ENR over one year, while 14CO2 production rates from [piperazine-2,3-14C]ENR were on the order of 7 to 30%. Similar rates obtained with cipro- and moxifloxacin indicated a limited utility of recording 14CO2 formation to prove FQ biodegradation. Quantitative biotransformation of ENR in two agricultural soils, a plant-derived compost, and cattle dung (t50 = 83 to 113 d) could only be determined by further oxidation. Upon further oxidation - provided labile compounds, concerns have been expressed on FQ biodegradability, long-term persistence, and potential selection of FQ-resistant zoonotic species in the environment. Metabolic transformation of enrofloxacin (ENR), a veterinary FQ, is limited to glucuronidation and partial oxidation of its amine substituent. In the only soil degradation study yet published (1997), 20.6% of the 14C label applied with [2-14C]enrofloxacin could be recovered as 14CO2 after 80 days, prompting our work on the fate of ENR in agricultural matrices.

**Results:**

1. Determination of TXA concentrations is very important for the establishment of a therapeutic drug. It is active against various cancer and immunologic diseases, including rheumatoid arthritis and psoriasis; it has the broadest dose-range. It can be given by many different routes; it is a cytostatic with a special position in anticancer chemotherapy. It is thus recommended as the drug of choice for measuring the determination of its concentration in blood serum and cerebrospinal fluid; it is the drug which has an antitet (leukocyt). Efficient and safe therapy requires broad knowledge of its mechanism of action, pharmacokinetics, toxicity, as well as interaction between simultaneously administered drugs. The therapy with this drug is very effective but it also has severe side-effects. The main reasons for discontinuing the treatment. Many authors have been trying to find risk factors of its toxicity.

2. Metabolism of TXA was carried out in 206 patients, 112 children and teenagers with acute lymphoblastic leukemia (ALL), non Hodgkin’s lymphoma (NHL) and osteosarcoma. 49 adults with rheumatoid arthritis; 65 healthy adults. TXA concentrations in blood serum were determined by the fluorescence polarization immunnoassay applying TDX Abbott analyzer. The following pharmacokinetic parameters for methotrexate were calculated: elimination rate constant, biological half-life time, apparent clearance, area under the the serum concentration-time curve. Determination of MTX toxicity was based on analysis of biochemical laboratory tests that characterised the function of the liver, the kidney, hematopoietic system and clinical symptoms. Activity of N-acetyl-beta-D-glucosaminidase (NAG), as an early marker of tubular epithelial damage, was used for measuring its concentration in blood serum and cerebrospinal fluid; it is the drug which has an antitet (leukocyt). Efficient and safe therapy requires broad knowledge of its mechanism of action, pharmacokinetics, toxicity, as well as interaction between simultaneously administered drugs. The therapy with this drug is very effective but it also has severe side-effects. The main reasons for discontinuing the treatment. Many authors have been trying to find risk factors of its toxicity.

3. The patients at high nephrotoxicity risk, who need special care.

4. MTX monitoring therapy in patients with rheumatoid arthritis.

5. MTX monitoring therapy in patients with rheumatoid arthritis easier in the patients exposed to toxic effects connected with delayed MTX metabolism in patients with rheumatoid arthritis.

6. MTX monitoring therapy in patients with rheumatoid arthritis easier in the patients exposed to toxic effects connected with delayed MTX metabolism in patients with rheumatoid arthritis when compared to corresponding values in the control group of healthy subjects.

**Conclusions:**

1. Determination of MTX concentrations is very important for the optimal establishment of therapeutic treatment in cancer patients. It could lead to making therapy easier in the patients exposed to toxic effects connected with delayed MTX elimination from the organism. 2. MTX monitoring therapy in patients with rheumatoid arthritis does not significantly improve the effectiveness of the treatment, but it can play an important role in increasing the safety of this drug.

2. The necessity of detailed estimation of kidney secretory function before the beginning and after the end of MTX therapy in the rheumatoid arthritis patients exists. That procedure may be helpful for the recognition of the patients at high nephrotoxicity risk, who need special care.
Decoding infections - How to use "administrative" data for clinical quality improvement

WILKE MH

In many countries vast data collections are existing, which all – more or less – are containing coded patient informations. These data collections are mainly used for administrative purposes especially in the DRG (CaseMix) settings they are used for funding, reimbursement, planning, etc. On the other hand the data contain – at least if the respective country is 'mature' in CaseMix – multiples of clinical and medical information. In some countries even medication information (using ATC-codes) such as France or the U.S. are collected and stored. Producing an increase in nociceptive responses to hindpaw formalin injection while NPY-sap both reduce sensitivity on the hotplate and to formalin in rats. In rats and dogs, SP-sap, at doses that produce significant anti-nociceptive effects, produces little or no evidence of toxic or systemic toxicity.

Methods: Neuropeptides, such as substance P (SP), dermorphin (mu opioid peptide), neuropeptide Y (NPY) and galanin, can be used to target the rhabdomyolysis inactivating protein, sarcoplasm, to neurons expressing the respective cognate receptors by conjugation through a disulfide bond. The peptide vectors bind to the corresponding receptors and are internalized taking sarcoplasm into the target cells. Rats are injected via temporary lumbar intrathecal catheters. After two weeks, the rats undergo nociceptive testing followed by immunohistochemical staining of spinal cord sections for the appropriate peptide receptors to assess loss of target neurons.

Results: Lumbar intrathecal injections of SP-sap, dermorphin-sap, NPY-sap and galanin-sap selectively destroy superficial dorsal spinal horn neurons that express the corresponding receptors but have no effect on dorsal root ganglion neurons. SP-sap in rats transiently reduces hotplate reflex responses, but produces long lasting reduction in operate escape responses to aversive heat and cold and nociceptive responses to hindpaw formalin injection while preventing/reversing hyperalgesia and allodynia due to nerve injury or inflammation. Dermorphin-sap destroys aminergic II mu opioid receptor-expressing neurons on the other hand the data contain – at least if the respective country is 'mature' in CaseMix – multiples of clinical and medical information. In some countries even medication information (using ATC-codes) such as France or the U.S. are collected and stored. Producing an increase in nociceptive responses to hindpaw formalin and reducing analgesic potency of intrathecal NPY-sap and, while NPY-sap both reduce sensitivity on the hotplate and to formalin in rats. In rats and dogs, SP-sap, at doses that produce significant anti-nociceptive effects, produces little or no evidence of toxic or systemic toxicity.

Conclusions: Molecular neurosurgery with targeted toxins is a powerful research tool in neuroscience and the "Magic Bullets" SP-sap, dermorphin-sap, NPY-sap and galanin-sap have great promise as a new approach to treatment of chronic pain based on targeted ablation of key populations of spinal dorsal horn nociceptive neurons via receptor-mediated endocytosis of the neuropeptide-saporin conjugates.

Investigation of Dibenzyl Trisulphide (Dts) Isolated from Petiveria Alliacea as an Immunomodulator with Cytotoxic/Anti-Proliferation Activity

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Background: Cancer is considered to be one of the leading cause of death. The need to find effective and safe therapeutic agents for treating cancers has been one of the greatest challenges for mankind. Thus, 70% of all human cancers such as breast, small cell-lung, malignant melanoma, ovary and colon have remained refractory or untreatable. To date, the most effective drugs known for treating malignant diseases are derived from natural products and their associated derivatives.

Methods: The following molecular biological techniques; cell culture, immunostaining, staining of F-actin, SDS-PAGE and Western blotting, tyrosine phosphorylation, proliferation/viability assay and one-dimensional (1D) NMR interaction studies were conducted according to Rosner et al., (2001). Animal studies using mice, were employed for evaluating the cell biological (cytotoxicity/anti-proliferation) and toxic effects of dibenzyl trisulphide (DTS) isolated Petiveria alliance.

Results: Dibenzyl trisulphide increased the weight of the thymus and Peyer’s patches via cell proliferation. The trisulphide has a dose-dependent anti-proliferation effect on the following human cancer cell lines: SH-SYSY neuroblastoma, MCF-7 mammary carcinoma, IPC-melanoma, A549 small cell lung cancer and 5637 primary bladder carcinoma. The SH-SYSY neuroblastoma cells were the most susceptible to DTS with an IC50 of 0.43 µM. Binding DTS to serum albumin in buffered saline enhanced its cytotoxic effects by 2500 on the neuroblastoma cells. Dibenzyl trisulphide had little or no toxic effects on the non-cancer cell line, HOFA. The mode of action found for DTS is an attenuation of the dephosphorylation of tyrosyl-residues of mitogen activated protein (MAP) kinases (ERK 1 and ERK 2).

Conclusion: The data obtained for DTS suggest that the compound could be an interesting prototype for investigation as an anti-cancer agent.

Counteracting drug resistance by nutritional management and genetic improvement of disease resistance - lessons from in-silico studies for nematode infections of sheep

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Background: Gastrointestinal parasitism (GIP) is a major challenge to animal production worldwide. Parasite resistance to drugs impedes the control of the disease and raises the need for alternative methods, such as host nutrition and breeding for resistance. Field study results on the efficacy of the two methods are however contradictory and give rise to much speculation. Aims: (1) to explore, in silico, the interactive effect of host genotype and nutrition on performance and GIP and relevant genetic parameters of sheep infected with nematode parasites. (2) to determine under what circumstances nutritional control and/ or selective breeding become feasible alternatives to chemophylaxis.

Methods: A mechanistic model, describing the growth, utilization, development of immunity and levels of nematode infections in lambs challenged with GI parasites was developed. Host genetic variation was assumed in the ability to grow and to resist GI parasites. Simulated breeds differing in growth and resistance genotypes, were infected daily with a trickle challenge of 3000 L3s of Teladorsagia circumcinta nematodes and given access to either good or poor quality grass. Results: Mean values for production and resistance traits 50 days post infection are shown in the table. Means, heritabilities (fraction of variability due to genetic variation) and correlations for growth and resistance traits changed markedly over time. Correlation estimates for growth and resistance traits were genetically related.

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Conclusions: Nutritional management and selective breeding are valuable alternatives to anthelmintics in the control of GIP, but their efficacy depends on the interaction between host genotype and the nutritional environment.

Reloading Ehrlich’s Magic Red Bullets: Targeting the Red Achilles Heel of Melanoma Using Phenothiazinium Dyes

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The use of red dyes as vital stains and metabolic probes was pioneered by Paul Ehrlich, who associated their cytotoxicity with spontaneous autooxidation of the bioreductively generated tufo-oxid. Altered redox signaling and regulation in cancer cells present a chemical vulnerability that can be targeted by selective chemotherapeutic intervention. Here, we demonstrate that 3,7-diaminofluorescein-based redoxcyclers (PRC) including methylene blue and toluidine blue O induce selective cell cancer apoptosis by NAD(P)H:quinone oxidoreductase (NQO1)-dependent bioreductive generation of cellular oxidative stress. Using PRC lead compounds against human metastatic G361 melanoma cells, apoptosis occurred with phenothiazine-autoxidation, loss of mitochondrial transmembrane potential, cytochrome C release, caspase-3 activation, and massive ROS production. Consistent with reductive activation and subsequent redoxycling as the mechanism of PRC cytotoxicity, co-incubation of human melanoma cell lines expressing PRC-cytotoxicity. In contrast, human A375 melanoma cells were resistant to PRC-induced apoptosis, and PRC-sensitive G361 cells were protected by preincubation with the NQO1-inhibitor dicoumarol. Indeed, NQO1 specific enzymatic activity was nine fold higher in G361 than in A375 cells. The critical role of NQO1 in PRC-biocytotoxicity and cytotoxicity was confirmed, when NQO1-transfected breast cancer cells stably overexpressing active NQO1 displayed strongly enhanced PRC-sensitivity as compared to vector-control transfected cells with base line NQO1 activity. Based on pilot studies performed in mouse xenograft models and the known overexpression of NQO1 in various tumors including melanoma and lung cancer these findings suggest the feasibility of developing PRC lead compounds into tumor-selective bioreductive chemotherapeutics. Supported in part by grants from NIH (R01CA122484; ES06894) and ABRC (0721).

Conclusions: Human epidermal growth factor (hEGF) is a 53 amino acid polypeptide found in our duodenum and salivary glands. It is capable of stimulating cell proliferation and differentiation of various epithelial tissues and has been reported previously. Applications of recombinant human epidermal growth factor in the treatment of hard-to-heal wounds

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Background: Human epidermal growth factor (hEGF) is a 53 amino acid polypeptide found in our duodenum and salivary glands. It is capable of stimulating cell proliferation and differentiation of various epithelial tissues and has been reported previously. Further studies of the applications of hEGF in the treatment of other hard-to-heal wounds, including drug-induced Steven Johnson syndrome, scalded skin, surgical wounds, and poririasis all resulted in an enhanced healing effect. The results support our view that topical application of hEGF, when administered at an effective dosage, may offer a simple and effective treatment to the management of various skin wounds.

Conclusions: 1) Topical application of hEGF-containing cream to skin wounds helps enhance the healing effect and reduce the healing time. 2) Topical application of hEGF presents a simple and effective treatment to the management of a broad range of skin wounds.

Feasibility of Mapping Brain pH Using 31P MR Spectroscopy

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Background: Magnetic resonance (MR) spectroscopy is a valuable method for the noninvasive investigation of metabolic processes. Although brain adenosinenuclearphosphate (ATP) studies can be found in multi-voxel 31P MR spectroscopy, previous studies of intracellular brain "potential of hydrogen" pH were conducted in single-voxel 31P MR spectroscopy. Aim: To explore the feasibility of mapping brain ATP and brain pH by using multivoxel 31P MR spectroscopy.

Methods: Phantom studies were carried out using a GE 3T scanner. Many available sequences were tested using phantom and the two dimensional (2D) RE/Soled MR Spectroscopy Chemical Shift Imaging (PRESS/CSI) sequence was selected because of better signal to noise ratio. Time of repetition (TR) was 1000 msec and time of echo (TE) 144 msec with 128 scan averages. The acquisition matrix was 16 x 16 phase encodings over a 24-mm field of view (FOV). Slice thickness was 10 mm. Then a healthy volunteer from MR research team was studied. Data were processed offline using the Spectroscopic Analysis of General Electric / Interface Definition Language (SAGE/IDL) software. Baseline and phase corrections were performed. Multivoxel spectra and brain ATP map were analyzed. Brain pH values were calculated from the difference in chemical shifts between inorganic phosphate (Pi) and phosphocreatine (PCr) resonances. Color scaling map was generated using Matlab software.

Results: Multivoxel 31P spectra were obtained for phantom and the healthy volunteer. PCR map was obtained in phantom. At this moment, peaks of Pi were not homogeneous in phantom studies. There was noise for multivoxel 31P spectra in volunteer study. Phosphohomocreatine (PME) peak, Pi peak, phosphocholesterol (PDE) peak, PCR peak, ATP peak, PiATP peak, and (PiATP) peak can be identified. Preliminary brain ATP map and brain pH map were generated in the volunteer.

Conclusions: It is feasible to map brain ATP and brain pH using multivoxel 31P MR spectroscopy. However, endeavors should be made to improve quality of multivoxel 31P MR spectroscopy.

The G-rich promoter and G-rich coding sequence of basic fibroblast growth factor are the targets of thalidomide in glioma

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Abstract: Although the high risk of teratogenicity, thalidomide is emerging as a treatment for cancer and inflammatory diseases. In recent years, several studies have shown the increased expression of basic fibroblast growth factor (bFGF), a potent angiogenic factor, in gliomas. The G-rich promoter and G-rich coding sequence of bFGF may be the targets of thalidomide. To examine whether thalidomide preferentially interacts with the G-rich coding sequence of bFGF, we measured the UV-VIS hydrolysis of thalidomide, Mei and Wu found that low concentration of thalidomide as 0.1 µg/ml target the G-rich region of bFGF in glioma. Earlier clinical studies have found that patients responding to this drug had high plasma levels of basic fibroblast growth factor (bFGF). This finding is consistent with the hypothesis that thalidomide down-regulates the transcription of bFGF in glioma. In addition, the expression of bFGF is closely related to the progression of glioma. The present study was to determine the possible molecular mechanism of thalidomide in glioma.

Materials and methods: To examine the influence of thalidomide, we used U-87 MG cells, which are a glioma cell line. First, we treated U-87 MG cells with different concentrations of thalidomide for 24 hours. After 24 hours, the absorbance at 230 nm was measured. In addition, the absorbance at 230 nm was quenched to a greater extent on thalidomide treatment. To examine the effect of thalidomide, we treated U-87 MG cells with different concentrations of thalidomide for 24 hours. After 24 hours, the absorbance at 230 nm was measured. In addition, the absorbance at 230 nm was quenched to a greater extent on thalidomide treatment. To examine the effect of thalidomide, we treated U-87 MG cells with different concentrations of thalidomide for 24 hours. After 24 hours, the absorbance at 230 nm was measured. In addition, the absorbance at 230 nm was quenched to a greater extent on thalidomide treatment.

Results: Using U-87 MG cell lines, we found that thalidomide, especially when encapsulated in a liposome, down-regulated the transcription of bFGF, which was reported to regulate the expression of different isoforms. Western blot analysis was used to analyze the levels of the various bFGF isoforms. Because bFGF belongs to the FGF gene family and is a potent angiogenic factor, it may be a target for thalidomide. Thalidomide may act as a target for the regulation of bFGF in glioma.

Conclusions: We conclude that thalidomide may be a target for the regulation of bFGF in glioma. Further studies are needed to determine the possible molecular mechanism of thalidomide in glioma.
Targeted Immunotherapy of Cancer Through TCR Gene Transfer

XUE SA, GAO L, THOMAS S, HART D, XUE JZ, MORRIS E, STAUSS HJ

University College London, London, United Kingdom

**Background:** Conventional cancer therapies are limited by their toxicity and lack of specificity. To achieve targeted immunotherapy, we have targeted WT1's Tumour antigen (WT1) which is overexpressed in most leukaemias and many solid cancers, and isolated WT1-specific T cell receptor (WT1-TCR) genes. Human T cells transduced with WT1-TCR eliminated leukaemia cell lines in vitro and in a NOD/SCID mouse model. To facilitate the clinical application of TCR gene therapy, we have modified this WT1-TCR retroviral construct aimed at improving TCR functional activity and reducing its cytotoxicity.

**Methods:** WT1-TCR constructs were generated in a retroviral vector in which the woodchuck hepatitis virus-derived post-transcriptional regulatory element was deleted. To enhance desired pairing, a second disulphide bond was introduced between the TCR α and β chains and the human TCR constant domains were replaced with murine sequences. Following transduction, the functional activity of WT1-TCR engineered T cells was tested by 51Cr release cytotoxic T lymphocyte (CTL) assay and intracellular cytokine staining. After the engraftment of CD34+ leukaemia progenitor cells from a CML patient in NOD/SCID mice, adoptive immunotherapy was performed with WT1-TCR engineered patient’s T cells.

**Results:** We have generated WT1-TCR constructs with improved safety features and enhanced functional activities as determined by tetramer staining, CTL killing and intracellular cytokine staining. To mimic the clinical settings, we engrafted CD34+ leukaemia progenitor cells into NOD/SCID mice, followed by adoptive immunotherapy with the patients’ T cells transduced with either WT1-TCR or control TCR. We show that mice treated with WT1-TCR engineered CTL had a greater survival than the mice treated with control CTL. Analysis of the bone marrow showed that control mice had engraftment of leukaemia cells, while WT1 CTL treated mice did not. These data provide a solid basis for a phase I clinical trial.

**Conclusions:** 1) Genetic modification of TCR genes provides a way of generating safe, efficient reagents for clinical applications. 2) WT1-TCR transduction of patient’s T cells offers a simple and efficient way of producing tumour specific T cells for the treatment of leukaemias. This has important implications for treating other WT1-expressing cancers.
A cost-effective Haemophilus influenzae type b conjugate vaccine in combination with DTwP-Hep B vaccine for developing countries – A perspective

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2 Birla Vidyapeeth Hospital, Pune
3 Serum Institute of India Ltd., Pune

Background: Despite the availability of Hib conjugate vaccines, Hib remains a leading cause of meningitis and pneumonia deaths, worldwide. Administration of combination vaccines has greatly simplified immunization activities and also helped in additional vaccines being incorporated into immunization schedule. IAP reviewed data on combination vaccines and declared that addition of Hib/Hepatitis B vaccines to DTwP vaccine as safe and effective for primary as well as booster vaccinations. This study was conducted to assess the immunogenicity and reactogenicity of DTPw HB + Haemophilus influenzae type b conjugate vaccine manufactured by SII in comparison with Trivianix HB + Hibrix vaccine of Glaxo Smith Kline (GSK) in Indian Children aged 6-14 weeks.

Methods: 304 children aged 6-8 weeks at enrollment were equally randomized to receive three doses of 0.5 ml of either SII or GSK vaccine intramuscularly, with a gap of one month between each dose. Pre and Post vaccination IgG antibody titres were assessed by ELISA at an independent laboratory, using Good Laboratory Practice (GLP) guidelines. Reactogenicity was assessed from the frequency of adverse events recorded by parents on diary cards. Study was conducted as per International Conference on Harmonization - Good Clinical Practice (ICH-GCP) guidelines and Declaration of Helsinki.

Results: Of the 304 children enrolled in both the groups, Post-vaccination GMTs in SII group, were 2.78 IU/ml, 50.87 IU/ml, 1.34 IU/ml, 616.73 mIU/ml and 7.55 µg/ml for anti Tetanus, anti-Pertussis, anti Diphtheria, Anti HBs and Anti Hepatitis B respectively. Corresponding values in GSK group were 2.52 IU/ml, 48.28 IU/ml, 0.99 IU/ml, 463.12 IU/ml and 7.82 µg/ml respectively. Post-vaccination sero-protective antibody was 100% for all components, in both groups except Pertussis component, which was 96.06% in SII group and 95.4% in GSK group. Frequency of adverse events was higher with the first dose and thereafter reduced in both the groups. Common adverse events were pain (SII=42%, GSK=44%), redness (SII-15%, GSK-14%), swelling (SII-29%, GSK-30%) and fever (SII=41%, GSK-45%). No Serious Adverse Event was reported in the study.

Conclusion: 1) DTPw HB + Hib vaccine manufactured by Serum Institute of India Ltd., Pune was found to be safe, immunogenic and non-inferior to GSK vaccine 2) In our time course experiment, we tested whether the co-administration of IL-12 expression plasmid and leishmanial antigen will skew highly susceptible BALB/c mice to Th1 response and protect from leishmaniasis. In this study, we assessed that SII vaccine showed a significant level of protection and was non-inferior to the GSK vaccine. We are planning to conduct a large scale, randomized, controlled study with over 1000 children to further validate these results.

Abstracts

Noninvasively Evaluation on Human Stress Using Salivary Biomarker

YAMAGUCHI M

Iwate University, Morioka, Japan

Background: Saliva sampling has the advantage that it is noninvasive, making multiple sampling easy and stress free. Salivary amylose activity (SAA) can be a useful index of plasma norepinephrine concentration under a variety of stressful conditions, since it appears that increased sympathetic nervous activity is a major stimulus of salivary amylose secretion. In order to realize a hand-held monitor of the sympathetic nervous system, we fabricated a completely automated analytical system using a dry chemistry system.

Methods: The monitor consisted of a disposable test-strip and an optical analyser (130 x 87 x 40 mm; 190 g), which was incorporated within a automatic saliva transfer device. The test-strip consisted of a collecting paper and a reagent paper containing 2-chloro-4-nitrophenyl-4-O-P-D-galactopyranosylmaltoside (Gal-G2-CNPs) as a substrate for amylase. The collecting paper is directly inserted into an oral cavity, and approximately 30 µl of saliva is collected from under the tongue. When Gal-G2-CNPs is hydrolyzed by amylase, the hydrolyzed product (CNPs) develops a yellow color and the reflectance is measured by an optical device. The definition of one unit activity (U) per mass of enzyme is that this activity produces 1 µmol of the reducing sugar, maltose, in 1 min. The SAA by video viewing was observed in 64 healthy adults. The ethical committee approved the study.

Results: When this monitor was used, it took 30 s for saliva sampling, 30 s for saliva transfer and measurement, and a total of one minute was enough to measure the SAA. The calibration curve of the monitor was within a range between 10 and 140 kU/l showing a coefficient of R² = 0.97. With regard to reproducibility of the measured results for the saliva transfer volume of the same samples, the coefficient variation (CV) was 5.5%. In our time course experiment, the SAA level increased just after the beginning of stressful video viewing in 63 of 64 subjects, and immediately returned to the pre-sress level just after the end of the video viewing. On the other hand, the SAA level was significantly decreased by smoothing video viewing.

Conclusions: It was demonstrated that the manufactured monitor enabled a user to automatically measure the SAA with a high accuracy. The SAA measurement may provide information on human stress

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Conclusions: It was demonstrated that the manufactured monitor enabled a user to automatically measure the SAA with a high accuracy. The SAA measurement will be powerful tool for psychological research.

Solute Leishmanial Antigen and Plasmid Expressing Interleukin-12 Protects BALB/c Mice from Leishmaniasis major Infection

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Background: In murine leishmaniasis, the induction of the T-helper type 1 (Th1) response contributes to protection, whereas the induction of Th2 response makes the mice susceptible to infection. Interleukin-12 (IL-12) plays a crucial role in the diversification of immune responses to the Th1 type. In this study, we tested whether the co-administration of IL-12 expression plasmid and leishmanial antigen will skew highly susceptible BALB/c mice to Th1 response and protect from leishmaniasis.

Methods: The BALB/c mice were intraperitoneally injected with the combination of IL-12 plasmid which encode p35 and p40 subunits and soluble leishmanial antigen (SLA) 7 days prior to the challenge with lethal dose of parasite. The protective effect was assessed with the development of a Th1 response, as demonstrated by the enhanced level of antigen-specific interferon- (IFN- ) and dominant production of IgG2a in the serum. In contrast, the administration of empty plasmid plus SLA or IL-12 plasmid alone failed to protect the disease and shape the Th1 response. Furthermore, the protective efficiency induced by the vaccination was clearly prevented by the injection of anti-IL-12 mAb.

Results: The mice which received IL-12 expressing plasmid plus SLA completely healed and the parasite burden in the local lymph nodes significantly decreased. The cured mice retained long-term immunity, and were resistant to any subsequent rechallenge of the lethal dose of the parasite. The protective effect was associated with the development of a Th1 response, as demonstrated by the enhanced level of antigen-specific interferon- (IFN- ) and dominant production of IgG2a in the serum. In contrast, the administration of empty plasmid plus SLA or IL-12 plasmid alone failed to protect the disease and shape the Th1 response. Furthermore, the protective efficiency induced by the vaccination was clearly prevented by the injection of antithrombin-IL-12 mAb.

Conclusion: The IL-12 expression plasmid is thus an effective adjuvant for the elicitation of a protective Th1 response against leishmaniasis and is therefore, considered to be appropriate for vaccinations that require the induction of Th1 type immunity.

Abstracts

All abstracts are listed in alphabetical order of the presenting author.
Interfere IGF-I pathways to augment sensitivities of colorectal cancer cells to current therapeutic agents

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Current chemotherapy agents are not highly effective against disseminated colorectal cancers. One major contributor to the limited effectiveness of the treatment is cancer cell producing high level of IGF-I and IGF receptors which has been proven protecting a broad range of colon cancer cells from a variety of apoptosis challenges used by chemotherapy agents. The IGF system, therefore, has become an attractive molecular target for anticancer therapies. Based on IGF-I structure we have designed and synthesised a novel IGF type I receptor antagonist. The effect of the antagonist on human colon cancer cell proliferation was examined by a non-radioactive assay; the apoptosis was revealed by determining the activities of cellular caspases 3/7/8,9. The apoptosis pathways were investigated by examining the level of pro-apoptosis singling protein with Western blotting. The results showed that IGF-I receptor antagonist induce cancer cell apoptosis and inhibits colon cancer cell proliferation. The changes of Caspase 3/7,8 and 9 activities and different expression level of Bax in cancer cells after treatment with the peptide suggested that the extrinsic pathway may play an important role in IGF-I receptor antagonist induced apoptosis in colon cancer cells. Treating different type of colorectal cancer cells by combination of IGF-I receptor antagonists with current chemotherapeutical agents has shown that IGF-I receptor antagonists significantly augment sensitivities of colorectal cancer cells to current chemotherapeutical agents. All these data suggested that IGF-I receptor antagonists could be developed to a therapeutic strategy for colorectal cancer.

Immunogenicity of mucosally delivered Lactococcus lactis expressing a malaria protein in rabbits

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On-oral immunization is preferable to injections from the point of view of safety, ease of administration and compliance. The gram positive food grade bacterium Lactococcus lactis is a potential vehicle for delivering immunogens to the mucosal immune system. We have investigated the immunogenicity in rabbits of the Plasmidium falciparum merozoite surface protein MSA-2 expressed on L. lactis. The 819 bp coding sequence of Plasmidium falciparum MSA-2 was presented on the L. lactis in two forms viz. as an intracellular molecule and one covalently anchored to the peptidoglycan layer of the cell wall. The recombinant L. lactis were delivered orally or intranasally, followed by 1 week of rest, and three or four peptides that had been positive in pre-existing peptide-specific immune responses in the circulation (personalized peptide vaccine) were injected subcutaneously in the femoral area once a week. The objective response rate was assessed according to RECIST.

Results: Twenty patients from 2 institutions were enrolled in this study. The median age was 63 years (range: 41-80 years). The PS was 0 in 10 patients (50%) and 1 in 10 patients (50%). The efficacy and toxicity were analyzed in 20 patients who received at least one course (8 weeks) of immunotherapy. Although no complete response was seen, a partial response was achieved in 5 patients, resulting in an overall response rate of 25%. Eleven patients (55%) had stable disease. The median overall survival was 9.0 months with a 1-year survival rate of 33%. The major grade 3-4 toxicities using CTCAE criteria were neutropenia (25%), anemia (20%), thrombocytopenia (20%) and anorexia (5%). All 17 dermatologic reactions at the vaccination site were scored as grade 1 or 2. No treatment-related deaths occurred during the study. Augmentation of peptide-specific CTL responses in the post-vaccination peripheral blood mononuclear cells was observed in 75%, while increased titer of peptide-specific IgG antibodies was observed in the post-vaccination plasma in 85%.

Conclusions: This study could be recommended for further stages of clinical trials because of safety, boosting of immune responses, and potential clinical benefits.
Aims: To analyse the changing Indian vaccine policies and practices with reference to 1) the roles of public and private sectors 2) adoption of new vaccines 3) Individual vs. Combination vaccines 4) National priorities and International pressurises.

Methods: Historical, economic and policy analyses based on various official documents, literature and field surveys. 

Results: Over the last two decades, Indian vaccine scenario has witnessed: 1) a drift away from the policy objectives of self-reliance, 2) declining role of the government and public sector and increasing role of the private sector in production and policy; 3) widening demand-supply gaps in primary vaccines due to private sector’s emphasis on new and expensive vaccines 4) adoption/promotion of new vaccines without adequate epidemiological proof of their need and cost-benefit analysis; 4) private sector using combination vaccines (combining universal and non-universal vaccines) to gain backdoor entry into universal vaccine markets. 5) emphasis on vaccine ‘coverage’ rather than on the ‘protection’ achieved, 6) overall vaccine strategies drifting along “supply push” rather than “demand pull” arguments and 7) ignorance or complacency in local government and lack of suitable policy support from international organizations to reverse these trends.

Conclusion: 1) Indian vaccine policies and practices are increasingly being driven by the market forces rather than evidence-based decisions, leading to shortage of affordable primary vaccines, proliferation of unnecessary new vaccines or their expensive combinations. 2) Immunization policies must be based on scientific principles and not by market vagaries. 3) The success of vaccination must be determined by protection achieved and not by money spent or ‘coverage’.
Background: ATP and adenosine are major regulators of energy metabolism maintaining optimum balance between oxygen supply and demand within the cardiovascular system. In addition to these fundamental roles, they are also potential biomarkers for cardiovascular protection, and as therapeutic targets for treatment of ischemic heart disease (HD), stroke, inflammatory diseases, cancer, and neurodegenerative diseases. The effectiveness of many cardiovascular drugs could be attributed to their effect on increasing plasma and tissue concentrations of adenosine and ATP. The main objective of our research effort in this program is to determine whether or not adenosine and ATP concentrations in plasma and RBC are useful surrogate biomarkers for development of cardiovascular agents in vivo.

Methods: Male New Zealand White rabbits (4 – 6 kg), normotensive SD rats and hypertensive SHR (325 – 400 g) were used for these investigations. They were treated with a known cardiovascular drug or normal saline (control), and kept either in a restrainer or placed on a research treadmill for an exercise test for blood sample collection and hemodynamic recordings. Blood samples were collected via an indwelling catheter from a carotid artery and immediately mixed with a stopping solution to stabilize adenosine and ATP during subsequent processing. Plasma concentrations of adenosine and red blood cell (RBC) concentrations of ATP and their metabolites were measured by a previously validated HPLC. Data between groups were compared by ANOVA and differences considered significant when p < 0.05.

Results: We have found that many cardiovascular agents block the uptake of adenosine by RBC, and that some of them enhance the hemodynamic effects of adenosine in the rabbit model. In addition, we have found that SHR have higher plasma concentrations of adenosine and RBC concentrations of ATP than the normotensive SD rats, and that exercise increases the concentrations of these biochemical regulators particularly in rats treated with the cardiovascular agents.

Conclusion: Many cardiovascular drugs and perhaps also drugs affecting energy metabolism could modulate the circulatory concentrations and cardiovascular effects of Adenosine and ATP which could be exploited for development of novel drugs and innovative therapies. (Supported in part by a grant-in-aid from CIHR/NShRP/PF/EF Regional Partnership Program).

Fluoroquinolones in the Treatment of Tuberculosis: Current Status and Future Issues

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In the 1980s to 1990s, fluoroquinolones were shown to have significant activities, both in vitro and in the murine model, against Mycobacterium tuberculosis. Later on, the clinical activity of fluoroquinolones in treating both drug-resistant and drug-susceptible tuberculosis, was demonstrated in a new largely uncounounded chemotherapy trials. The utility in the former setting became rather important due to the surge in multi-drug resistant tuberculosis globally in the past two decades. In parallel, fluoroquinolones were also found to have a useful role in treating patients with significant intolerance to conventional antituberculosis drugs. Furthermore, in order to improve patient adherence and curtail development of drug resistance, a genuine need arose to discover more efficacious drug regimens to shorten the six-month duration of standard antituberculosis chemotherapy for drug-susceptible disease. This prompted focused research on the sterilizing capacity of fluoroquinolones against M. tuberculosis persists in disease lesions, especially regarding that of the newer fluoroquinolones. Animal experiments have apparently demonstrated the potential ability of these new agents in shortening the duration of therapy, in comparison to standard short-course treatment, and producing stable cure of disease without relapse. Controlled clinical studies are now ongoing to evaluate these issues in patients with tuberculosis. Some preliminary results appear rather promising. However, the emergence of resistance against fluoroquinolones in M. tuberculosis strains, often in the deadly form of extensively drug-resistant tuberculosis would pose a great concern regarding the effectiveness of these new agents in treatment of the disease. Novel strategies are thus required to circumvent this challenge.

Pharmacokinetic and Drug Metabolism: A Bioanalytical Perspective

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Drug development is a complex process that requires technical and scientific expertise in many area and contributes to the commercial success in a significant way. The modern drug development process is not only a technical one but a scientific one as well. It requires the contributed knowledge from pharmaceutical chemistry, pharmaceutics, drug metabolism, pharmacokinetics, medicinal chemistry, and cell biology. In this presentation, our focus is on pharmacokinetics and drug metabolism and their role in current drug development process. There is a recent trend of moving towards patient-specific or personalized medicine. The understanding of pharmacokinetics and metabolism is essential for understanding the characteristics of a new molecule entity and is necessary for a rational drug development program. Among these processes a key component is the development of bioanalytical methods needed to accurately quantify biological samples of administered drugs. This is an important factor in the study of the relationship of dose, exposure and effect, and reaching reasonable PK/PD conclusions regarding the drug safety and efficacy. A fundamental flaw in drug development program would be the lack of reliable effectiveness and safety profiles and rich database to support pivotal dose regimen design for the clinical trials. Validated bioanalytical assays ensure accurate information regarding PK/PD properties and further the establishment of informative dososequence (PK)-response (PD) relationship throughout the drug development program, and eventually provide the best possible foundation relevant to more efficacious and safer therapeutic use of the drug in man.

Antineoplastic effects of an aurora B kinase inhibitor against breast cancer

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Aurora B kinase is an important mitotic kinase involved in chromosome segregation and cytokinesis. It is overexpressed in some human breast cancer as well as other cancers, and has been linked to genetic instability, tumorigenesis and invasive disease. Aurora B is an important molecular target for chemotherapeutics, and several small molecule inhibitors of Aurora B are now in clinical trials. AZD1152 is a dihydrogen phosphate prodrug which is converted in the serum to AZD1152-HQPA (hydroxyquinazoline pyrazol anilide). AZD1152-HQPA is a small molecule ATP binding pocket competitor that selectively inhibits Aurora B (Ki = 0.36 nM) compared with Aurora A (Ki = 1369 nM) and 50 other kinases. AZD1152 has been shown to have antineoplastic activity in acute myelogenous leukemia, multiple myeloma and colorectal cancer. To date, AZD1152 has not been evaluated in human breast cancer, the leading cancer among US women.

We investigated the effect of AZD1152 on breast cancer cells. AZD1152-HQPA inhibited a panel of human cell lines including Her18, a Her-2 overexpressing breast cancer line. The IC50 for these lines were 14-125 nM and in the same range as reported by others. Time-lapse photomicroscopy of Her18 cells treated with AZD1152-HQPA showed enlarged multicellular clusters. Micronuclei and chromosome bridges were observed. FACS analysis demonstrated polyplody consistent with the increase in DNA copy number in multicellular cells. AZD1152-HQPA treatment resulted in decrease in Aurora B activity (as indicated by decrease in phosphorylated Histone H3), mitotic failure and apoptosis (as indicated by Annexin V-FITC FACS, increases in PARP cleavage and increase in Bax). AZD1152-HQPA inhibited the clonogenic potential of Her18 cells. AZD1152 (62.5 mg/kg/day on days 1 and 2 of a 7-day repeating cycles) also demonstrated significant antineoplastic activity against Her18 xenografts in nude mice. Reduction in phospho histone H3 was demonstrated by immunoblotting in tumor samples from the AZD1152-treated group. Bax was increased in the AZD1152-treated tumors. Immunohistochemistry for Ki-67 and activated caspase 3 showed that AZD1152 reduced proliferation and increased apoptosis in the treated xenografts.

In conclusion, these data suggest that AZD1152 may be an effective chemotherapy agent for human breast cancer. Further investigation and possibly a clinical trial in breast cancer patients are warranted.
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The fecundity of Schistosoma japonicum was impaired by administration of low dose cyclophosphamide

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The maintenance of the balance between excitation and inhibition in the brain is essential to avoid pathological consequences. γ-amino-butyric acid (GABA) is the principal source of inhibition, it uses glutamate (the principal excitatory transmitter) to block over-excitation in the synapses of the brain and thus preventing seizures. GABAergic agents that enhance inhibition in the brain are anticonvulsant and anxiolytic. A dilemma arises during brain development when a mismatch between the strength of GABA and glutamatergic synapses which may prevent growth and synapse formation and perturb the sculpting of neuronal circuit connections, if this condition prevails, there will be a failure of neuronal communication and may contribute to disorders in information processing in autism. Therapeutic drug design based on drug-receptor interaction becomes more challenging in disorders of developmental neurobiological origin involving multiple signalling processes such as autism. The discovery of drug development program in autism will require the application of basic science research with therapeutic modalities that can address functional imbalances in the homeostatic mechanisms of the disorder. A circuit-centered approach to drug target design for developmental neurobiological disorders becomes more challenging in disorders of developmental neurobiological origin involving multiple signalling processes such as autism. The discovery of drug development program in autism will require the application of basic science research with therapeutic modalities that can address functional imbalances in the homeostatic mechanisms of the disorder.

Methods: This study included 72 C57BL/6J female mice, which were divided into control group, single treating group and consecutive treating group (24 mice per group). The percentage of CD4+CD25+ Treg cell in peripheral blood and spleen of three mice from each group were examined by FACS per normal salt solution (NS). Mice were infected with 30±1 cercaria of S. japonicum at 3-weeks of age. Mice were then treated with low dose cyclophosphamide (CTX) at 12-weeks of age (12 mg/kg) and sacrificed at 12 weeks by cervical dislocation. The fecundity of S. japonicum were determined by UV-vis.

Results: The percentage of CD4+CD25+ Treg cell in peripheral blood and spleen of mice was reduced significantly both in single treating group and in consecutive treating group (24 mice per group, weight: 20±5g). The mice in single treating group were given a single low dose of CTX (50mg/Kg) by i.p., mice of consecutive treating group were given a dose CTX per week by i.p., and mice of control group were given placebo of normal salt solution (NS). Mice were infected with 30±1 cercaria of S. japonicum at seventh day after first injection. The level of CD4+CD25+ Treg cell in peripheral blood and spleen were reduced significantly in single and consecutive treating group compared to control (mean ± S.EM for controls 1.54 ± 0.08, autistic 0.92 ± 0.09). Significant damage to such impregnated tumor cells is observed in comparison with cisplatin and mitomycin c. The lack of cytotoxicity of FeP-liposome is reported. A lack of cytotoxicity of FeP-liposome and an efficient generation of toxic OH from O{sub2} through the iron-catalyzed dismutation and the Fenton reaction allow tumor cell killing where the O{sub2} concentration is locally increased as a result of reduced activity of SOD and catalysts in the cells.

Methods: Lewis lung carcinoma tumor cells were treated with some drugs (0 to 100 μM). Cytotoxicity was determined by staining the cells with Alamar blue to obtain the effective concentration (EC{sub50}) of drug required to produce 50% lethal dose against cell. Rate constant (k{subcat}) of the catalyzed O{sub2} dismutation was analyzed by stopped-flow method. Particle size ( ) of liposome was measured by DLS and TEM. The relative hydrogen peroxide resistance against SOD (H{sub2}O{sub2} resist) of FeP was determined by UV-vis.

Results: Significant damage to such impregnated tumor cells is observed in comparison with cisplatin and mitomycin c. The lack of cytotoxicity is shown in control experiments using SOD and manganese-porphyrin (MnP) in place of FeP, which suggests that the cell damage is induced by OH, considering that SOD and FeP only show O{sub2} dismutation but do not participate in Fenton reaction. The k{subcat} of FeP-liposome is analogous to that of SOD, which also indicates that has a potential to act as catalyst of O{sub2} dismutation. The FeP-liposome is porphyrin-doped nanoparticle and has a strong H{sub2}O{sub2} resistance. The properties of the liposome will permit its continuous circulation in blood, porphyrin-based tumor targeting, and accumulation in a pathological site such as tumor tissue.

Conclusions: We have demonstrated that FeP-liposome bearing SOD-like and Fenton reaction activities present a potential target for design and development of novel anticancer drug. Our approach opens the door for liposome-loading and tumor-targeting of a much expanded selection of metalloporphyrin.

All abstracts are listed in alphabetical order of the presenting author.
Incorporation of Paclitaxel into Well-Defined Amphiphilic Block Copolymer
Micelle Having Phospholipid Polymer Sequence

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Background: Paclitaxel (PTX) is one of the most effective and commonly used drug for the treatment of various cancers. Due to the poor solubility of PTX in water and in many other acceptable pharmaceutical solvents, a specific solubilizing agent such as Cremophor EL is used to formulate PTX in a commercial injection solution. However, serious side effects have been reported. Therefore, a much safer solubilizing reagent has been needed. To realize safer and effective drug administration, novel well-defined and biocompatible micelles of amphiphilic diblock copolymers containing Phospholipid polymer sequences were synthesized.

Methods: At first, the homopolymer of 2-methacryloyloxyethylphosphorylcholine (MPC) was synthesized in water by reversible addition-fragmentation chain transfer (RAFT) controlled radical polymerization. Using this MPC homopolymer, AB type diblock copolymers of n-butyl methacrylate (BMA) were synthesized. Association behaviors of the amphiphilic diblock copolymer (pMPCm-pBMAN) with varying pBMA block lengths were investigated by nuclear magnetic resonance (NMR), fluorescence probe, static light scattering and quasi-elastic light scattering measurements. A given amount of PTX was dissolved in ethanol, and the PTX solution was added to an aqueous solution containing various amounts of the block copolymers. The solubility was evaluated by the transparency of the solution.

Results: Fluorescence spectra of the probe indicated that the probe was solubilized in polymer micelles in water. The formation of polymer micelles comprising a core with pBMA blocks and shell with hydrophilic pMPC block was confirmed by NMR and light scattering data. The size and mass of the micelle increased with increasing pBMA block length. PTX dissolved well in aqueous solutions of the block copolymer as compared with pure water, implying that PTX is incorporated into the hydrophobic core of the polymer micelles.

Conclusions: Well-defined amphiphilic block copolymers composed of pMPC and pBMA blocks were prepared via RAFT controlled radical polymerization. The amphiphilic block copolymer formed polymer micelles in aqueous solutions because of hydrophobic associations among pBMA block. By use of the diblock copolymer, the water solubility of PTX can be improved.
Methotrexate in Dermatology through 50 Years
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Background: Historical evaluation. Methotrexate (MTX) is a cytostatic, well established in treatment of malignancies. It is the oldest drugs used today for systemic therapy of psoriasis. 1958 was the start of MTX in dermatology. Originally it was prescribed daily for 5-10 days followed by a rest period of a few days. Since 1970 weekly oral or parental doses became the established way and together came trials of combination therapy. In 1978 it was found that MTX lead to more than 75% improvement in 90% of psoriatic patients.

Methods: Studies of mechanisms of action and establishing side-effects. Despite many years use of MTX, there are uncertainties concerning mechanisms of action. Among the many are cytokine inhibition (interleukin 1 and 6, and tumor necrosis factor (TNF alpha) and inhibition of neutrophil leukotriene and platelet activity factor (PAF). Besides inhibition of adhesion, immune-modulation, and aspects of anti-inflammatory properties, a main effect is induction of apoptosis in activated T-cells. In mid nineteen-seventies it was well established, that long-term usage of MTX could induce liver damage, which in some cases led to fibrosis or even cirrhosis. Decline in popularity of MTX followed due to a "liver scare", as it did due to the recommendations that liver biopsies be performed to monitor treatment. We do not recommend a pre-treatment liver biopsy unless one is dealing with a high risk factor for liver disease. Measuring aminotransferal propepy of type III procollagen (PIPPN) is preferable. This is a non-invasive marker of fibrogenesis, that can show that as long as PIPIn is normal, no significant development of fibrosis is taking place. Dermatologists are also in increasing numbers using MTX in cases of dermatomyositis, cutaneous sarcoidosis, scleroderma, pemphigus and pemphigus, Behcet’s disease, impetigo herpetiformis and mycosis fungoides. This will often be given in combination therapy as in psoriasis. Among the latter can be mentioned combinations of MTX with the TNF-alpha inhibitors etanercept or infliximab, where a large number of patients over several years have benefited.

Results and Conclusion: If the rules for accepted control are adhered to, the risk/benefit ratio for MTX should be considered one of the most valuable treatments in dermatology. The biologics are generally, but not always of higher dimension in relation to clearling.

Structural and Functional Diversity of Endogenous Antimicrobial Oligopeptides
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Background: Chemical substances named as oligopeptides consist of 2 to 50 amino acid residues. Natural oligopeptides may regulate nearly all vital processes. To date, primary structures of more than 1000 oligopeptides have been identified: 1) To elucidate all known natural antimicrobial oligopeptides; 2) To describe their structural and functional diversity; 3) To point their usage in medicine, veterinary, and food conservation.

Methods: This study was performed using our EROD-Moscow (Endogenous Regulatory OligoPeptides) database (http://erop.inbi.ras.ru). It contains complete information on natural oligopeptides.

Results: It has been shown that chemical structures of natural oligopeptides have been identified from more than 1000 different species representing all the biological kingdoms. More than 1500 oligopeptides possess antimicrobial functions. They are active against bacteria, fungi, viruses, stimulate antiviral and antimural resistance. These substances were found out in animals (1211), bacteria (109), fungi (162), plants (162), and viruses (2). It is known 121 natural oligopeptide structures. Primary structures of antimicrobial oligopeptides are characterized as having widely diverse sequences. Many of them display a net positive charge, ranging from +2 to +18. Nearly all antimicrobial oligopeptides form amphipathic structures upon interaction with target membranes and exhibit antimicrobial activity against a wide variety of micro-organisms. Antimicrobial oligopeptide structure and function offers hope for discovery and development of improved agents to prevent or treat infectious diseases caused by pathogens that resist conventional antimicrobial agents. Various human oligopeptides show a potent effect on pathogenic micro-organisms including antibiotic-resistant bacteria. Moreover, human defenses indicate that these oligopeptides are involved in various biological processes associated primarily with defensive and regulatory responses to infections by pathogenic agents.

Conclusions: Biomolecules (oligopeptides without any dangerous side effects due to their natural origin 1) can be used in therapy and veterinary; 2) can be considered as natural preservatives of food products. 3) are promising for hygiene and cosmetics.

Portal Haemodynamics and Plasma Transaminase Levels before and after Prostacyclin Analog Administration in Patients with Chronic Viral Hepatitis
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Background: Under physiological conditions, hepatic stellate cells produce prostacyclin to regulate the haemodynamics of portal microcirculation; in chronic liver disease, there is a progressive dysfunction of hepatic stellate cells and a reduction in prostacyclin production. The administration of a prostacyclin analog might optimize the regulation of hepatic stellate cell function in patients with chronic liver disease. Aims: 1) To evaluate the hepatic haemodynamics and 2) to monitor the inflammatory indexes before and after a prostacyclin analog infusion. Methods: 11 patients (6 males and 5 females; mean age 60.4±8.8 years) with histological diagnosis of chronic viral hepatitis were enrolled in the study after the informed consent document was signed by each-one. In each patient, before and after 3 days of physiological solution infusion (placebo) and before and after 3 days of prostacyclin analog infusion (at a dosage of 2 ng/Kg/min for 6 hours/day), serum levels of liver enzymes (GOT, GPT) were evaluated and, with color Doppler sonography, portal flow velocity (cm/s) and portal diameter (cm) were measured to calculate portal flow volume (PFV) and congestion index (CI).

Results: After prostacyclin analog infusion, patients had an increase in PFV (p<0.01) and a decrease in CI and GOT serum levels (p=0.01 and p=0.05, respectively), as compared with pre-prostacyclin analog infusion. No statistically significant difference was observed in GPT serum levels.

Conclusions: After 3 days of prostacyclin analog infusion, a significant amelioration of the hepatic haemodynamics (as demonstrated by the increase in PFV and the decrease in CI) and of the liver inflammatory indexes (as demonstrated by the decrease in GOT serum levels) was achieved. 1) All patients had a better portal liver perfusion. 2) Patients had an excellent decrease in liver enzymes while in only one patient their levels worsened. 3) The mean GOT transaminase serum levels were significantly lower relative to those of pre-prostacyclin analog infusion; 4) the liver function seems to benefit from prostacycin analog infusion, but prospective studies on a larger population are needed.
Neuropeptides in Stress-Related Disorders: Possible Interaction of Vasopressin and Corticotropin Releasing Hormone

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Background: Despite the great importance the treatment of affective disorders is not solved, yet. New drugs target neuropeptides, especially vasopressin (AVP) and corticotropin releasing hormone (CRH) as important regulatory molecules of the stress axis. Aims: 1) Summarize the available data on the role of AVP and CRH in affective disorders based upon their stress axis regulation. 2) Discuss the possible interaction of the two molecules.

Methods: This study will present our own data from AVP-deficient Brattleboro rats in scope of the literature. Behavioral studies (anxiety on elevated plus maze, depression-like behavior in the forced swim test etc.) are combined with analysis of the stress axis by plasma hormone content measurements (radioimmunoassay) and molecular biological methods (e.g. in situ hybridization).

Results: AVP-deficient animals are less anxious and show lower prevalence of depressive-like behavior in behavioral tests. These behavioral changes do not go parallel with changes in hormone levels. The CRH mRNA levels in the nucleus paraventricularis hypothalami are higher in AVP-deficient rats. Our results are supported by studies on high anxiety rats as well as by human data. The contribution of V1a or V1b receptors seems to be controversial. Moreover, several studies suggest that CRH rather than AVP is the key molecule of the process.

Conclusions: 1) AVP seems to be causally involved in the development of anxiety and depression. 2) The participating brain AVPergic circuits are distinct from those regulating the stress axis by plasma hormone content measurements (radioimmunoassay) and molecular biological methods (e.g. in situ hybridization).

New Development of Drug Solubilization

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Background: Approximately one of every two new drug candidates identified by high-throughput methods suffers from a very low water solubility, which usually give rise to poor or erratic bioavailability, and consequently these compounds are frequently not taken forward for further development. Therefore, water-solubilization of poorly soluble drugs is a considerable challenge for drug discovery and development. The commercially available polyethyleneoxide-based nonionic surfactants, such as Cremophor EL and Span 80, are used as drug solubilizers, but their applications are limited by their relatively low solubilizing capacity and serious side effects. Amides in conjunction with a hydrophobic chain were examined here because 1) amides should have greater solubilizing power than alcohols, ethers, etc; 2) amides are enzymatically hydrolyzable (yet chemically stable); and 3) amides, being both hydrogen-bond acceptors and donors, may improve the solubilization capacity by interacting with drug substances having hydrogen-bonding sites.

Methods: Twelve amide-based surfactants (“peptoads”) were synthesized through multi-step sequences, and their physicochemical properties were studied by X-ray analysis, solubility studies, surface tensiometry, and molecular dynamics simulations. One peptoad (namely peptoad G) was investigated for its capacity to solubilize eleven poorly water-soluble drugs by the shake-flask method, and the solubilizing mechanism was explored by molecular dynamic simulations. Additionally, the in-vitro toxicity of peptoads was evaluated on rat macrophage cell lines (RAW 264.7) by MTT assay.

Results: Peptoads possessing short carbon chains (seven carbons) are highly surface-active. Peptoad G can solubilize poorly water-soluble drugs very efficiently, by changing the water to drug ranging from about 20 to 1100-fold for eleven peptoads tested. Molecular dynamic simulations on solubilizing paclitaxel by peptoad G showed that peptoad molecules, hydrogen-bonded to the drug, surround the drug with hydrogen chains that embed themselves into the interior of peptoad “clumps”. It is by this mechanism that paclitaxel is solubilized. Additionally, the in-vitro toxicity of peptoad may be comparable to Cremophor EL and Tween 80.

Conclusions: The colloidal structure of peptoads, along with their solubilizing capacity on poorly water-soluble drugs, their unique solubilizing mechanism and their low toxicity, makes them promising drug-solubilizing agents.

Nasal Vaccination against Bacterial Toxins

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Background: Toxins produced by infectious bacteria often cause fatal diseases. Vaccines against bacterial toxins have been demonstrated as effective countermeasures against infectious diseases. However, with traditional immunizations by needle injection, there remain concerns about the availability of medical service and social acceptability. Our research aims to develop highly efficient and easily administered nasal vaccines against bacterial toxins.

Methods: This study tested a prototypical nasal vaccine against botulinum neurotoxin type C (BoNT/C). A replication-incompetent adenoviral vector encoding a human codon-optimized heavy chain C-fragment (H50) of BoNT/C was produced in the laboratory. It was evaluated as a mucosal vaccine against BoNT/C in a mouse model. Groups of mice were intranasally administered with single doses of adeno-viral vector Ad5/3-BoNT/C-H50, ranging from 101 to 2 × 107 plaque forming units (pfu). Serum and mucosal Anti-H50 antibody responses were measured by ELISA. The neutralization capacity of anti-sera from mice vaccinated with Ad5/3-BoNT/C-H50 was determined by in vitro toxin neutralization assay. The protective efficacy of the nasal vaccine was assessed by challenge with up to 107 × mLDF50 of active BoNT/C.

Results: Single intranasal inoculation of the Ad vector elicited a high level of H50-specific IgG, IgG1, and IgG2a in sera and IgA in mucosal secretions as early as 2 weeks after vaccination. The antigen-specific serum antibodies were maintained at a high level at least until the 27th week. Immune sera showed high potency in neutralizing BoNT/C, as indicated by in vitro toxin neutralization assay. The mice that received a single dose of 2 × 105 pfu of Ad vector were completely protected against challenge with up to 107 × mLDF50 of BoNT/C. The protective immunity showed vaccine dose-dependence from 105 to 2 × 107 pfu of adenoviral vector. In addition, animals that received a single intranasal dose of 2 × 105 pfu Ad vector could be protected against 100 × mLDF50 of BoNT/C after 27 weeks after vaccination. Animals with preexisting immunity to adenoviral vector could also be vaccinated intranasally and protected against lethal challenge with BoNT/C.

Conclusions: These results suggest that the adeno-viral vector is a highly effective gene-based mucosal vaccine against BoNT/C. This study has set up a platform to develop needle-free nasal vaccines against bacterial toxins.

Authors’ disclosure statement: This work was supported by the US Public Service research grant AI055946 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

The combined use of HPLC, Gel-LC-MS/MS and microarray in monitoring product quality and safety for gene therapy

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Gene therapy has a unique potential for treating genetic diseases and is now regarded as an indispensable addition in conventional medicines. Quality and safety assessment of gene therapy products presents a great challenge, because they are cell products as well as multi-gene products, including viral and therapeutic proteins and modified cells. In this presentation, we will review our collective experience of using a Gel-LC-MS-MS/MS analysis and the gene-array technology to evaluate quality of HPLC purified products and, to assess the contribution of a range of potential components presented in a final production in product potency and safety in vitro and in vivo systems. The identification of protein biomarkers, transcriptional and toxicogenomic markers using the advance Gel-LC-MS-MS/MS analysis and gene-array supports the development of robust and product-specific assays for monitoring the quality, potency and safety of complex products for gene therapy.

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EHRLICH II - 2nd World Conference on Magic Bullets
Celebrating the 100th Anniversary of the Nobel Prize Award to Paul Ehrlich
Nürnberg, October 3-5, 2008
Proline Specific Peptidases as New Targets for Drug Development: Pharmacology and Pathophysiology

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Background: Enzymes hydrolyzing peptide bonds involving proline are of particular interest because of the significance of this amino acid in the conformation of peptide chains. Recent studies have shown that prolyl endopeptidase (PE) and dipetidylpeptidase IV (DP-IV) participate in the metabolism of proline-containing neuropeptides related to pathogenesis of Alzheimer’s disease, Parkinsonism syndrome, muscular dystrophies and other degenerative diseases. The aim of our study was to investigate the antidepressive, antiamnestic and anhippoxic properties of PE and DP-IV inhibitors.

Methods: Forced swimming test was used for antidepressive activity studies. The investigation of age related mental decline, motor behavior and estimation of PE levels was carried out in different brain regions (cortex, hippocampus, hypothalamus) was carried out in Wistar rats of different ages (3 - 24 month old). Hypobrinc and acute hypoxic hypoxies were used for study DP-IV inhibitors.

Results: The administration of the PE inhibitors reduces the duration of immobility and potentiates apomorphine-induced stereotyped behaviors. Strong correlation between Ki values and antidepressive and antiamnestic activity of tested inhibitors were revealed. PE inhibitors mimicked the effects of antidepressants. PE level significantly rose in a brain rats 18- and 24 month old. A reduction of learning ability in the active avoidance test and a decline of performance in the reflex of passive avoidance by rats 16-24 month old was shown. Strong correlation between cognitive impairments, neurologic deficiency and brain PE level was found. All tested PE inhibitors protected animals from scopolamine-induced amnesia in a rat passive avoidance test. DP-IV inhibitor reduced animal mortality during hypobaric hypoxia, and injected intracerebrally changed ventilatory response to acute hypoxia.

Conclusions: 1) The pharmacological activities of PE and DP-IV inhibitors appear to be mediated via possible accumulation of neuropeptides in the brain. 2) The biochemical and pharmacological investigations of PE and DP-IV inhibitors may lead to appearance of a new class of psychotropic drugs with antidepressive, antiamnestic and anhippoxic properties.

Paroxetine-induced CYP2D6 Phenotype Conversion and Clinical Outcomes

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Background: Selective serotonin reuptake inhibitor paroxetine is often prescribed antidepressant in depressive and/or anxiety disorders. Metabolised largely by the polymorphic cytochrome P4502D6, paroxetine exhibits substantial variation in activity among patients. Paroxetine has an inhibitory effect on isoenzyme CYP2D6, this can result in a phenotype conversion to the poor metabolic (PM) phenotype. Paroxetine therapy may be associated with incidence of adverse effects due to an inhibition of its own metabolism. Aims: effect on drug toleration and drug efficacy during paroxetine therapy.

Methods: Subjects: Part A: Influence of paroxetine treatment on phenotype conversion from extensive metabolisers (EM) to PM (n=85). Part B: Influence of CYP2D6 phenotype on clinical response in acute paroxetine treatment (n=16). Part C: Influence of phenotype on the efficacy and the adverse effects in the maintenance paroxetine treatment (n=55). Dextromethorphan test was used for the determination of the metabolic activity of CYP2D6. Genotype determination of patients is based on the detection of polymorphisms in CYP2D6 gene exons 3, 4, 5, 6. The Hamilton Anxiety Scale (HAMA) and Clinical Global Impression-Severity of Illness Scale (CGI S) were used for clinical response. The Arizona Sexual Experiences Scale (ASEX) was used for sexual dysfunction examination.

Results: Part A: The effect of paroxetine treatment on CYP2D6 phenotype: in 55 patients sample 34 patients with homozygote EM genotype were found. In 20 of them, PM phenotype was assessed. Part B: In general, earlier onset of the effect of therapy in the acute study (n=16) was observed. Part C: No variation in therapeutic efficacy and adverse effects between PM and EM was found in the maintenance treatment (n=55). One unique output of the study is the variance in the incidence of sexual dysfunction depending on CYP2D6 activity in the females.

Conclusions: 1) PM phenotype patients are more vulnerable to the paroxetine treatment. 2) CYP2D6 activity testing is recommended in all cases with an unexpected therapeutic response. 3) Predicting sexual dysfunction based on the metabolic phenotype and gender during treatment with CYP2D6 inhibitors could be recommended.
Chemoembolisation Using Drug Eluting Beads: Magic Bullets Targeted by Arterial Guidance

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Transarterial chemoembolisation (TACE) is used in the treatment of hypervascularised tumours such as hepatocellular carcinoma (HCC) and involves delivering a drug into the hepatic artery followed by occlusion of the artery with an embolisation agent to starve the tumour of oxygen and nutrients. Although randomised studies have demonstrated a survival benefit for TACE (Llovet et al. 2002), the procedure varies vastly in clinical practice leading to inconsistent outcomes. Drug eluting beads (DEB) have been developed from sulfonate-modified polyvinyl alcohol hydrogel microspheres. These devices are capable of targeting outcomes. Drug eluting beads (DEB) have been developed from sulfonate-modified polyvinyl alcohol hydrogel microspheres. These devices are capable of targeting intra-arterial tumours with drugs by flow-directed delivery down the feeding arteries and subsequent embolisation of the tumour capillary bed. The DEB affect intra-arterial delivery of chemotherapeutic agents over a sustained period in a controlled manner; deliver a high concentration of drug local to the site of the tumour; reduce the delivery of chemotherapeutic agents over a sustained period in a controlled manner; subsequent embolisation of the tumour capillary bed. The DEB affect intra-arterial delivery of chemotherapeutic agents over a sustained period in a controlled manner; deliver a high concentration of drug local to the site of the tumour; reduce the systemic exposure to free drug; occlude the tumour arterial blood supply. DEB have been well characterised systemic exposure to free drug; occlude the tumour arterial blood supply. DEB have been well characterised and exhibit reduced systemic exposure (Hong et al. 2002), the procedure varies vastly in clinical practise leading to inconsistent outcomes. Drug eluting beads (DEB) have been developed from sulfonate-modified polyvinyl alcohol hydrogel microspheres. These devices are capable of targeting outcomes.

Possibilities of Nonconventional Application of Streptokinase: Studies at the Molecular and Cellular Levels

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Background. Traditionally the preparations of streptokinase (SK) are used in clinical medicine for dissolution of thrombi, fibrin deposits.

In 1987-1991, we described pronounced superoxide dismutase-like activity of SK in chemical systems of superoxide radical generation, the inhibition of plasminogen-activating ability of SK by scavengers of superoxide radical (nitro tetrazolium blue, adrenaline), nucleotides (ATP, cAMP and NAD at rather high concentration: ≥ 0.01 M).

In 1993 we proposed the SK-containing composition for the treatment of patients with long-nonhealing wounds (a patent of the Russian Federation). Its local application in several surgical clinics of Minsk has yielded good results – the healing of such wounds was achieved in all cases.

In 1991, instillation of the SK solutions in eyes was used for treatment of guinea pigs with experimental herpetic keratoconjunctivitis (a patent of the USSR).

Since 1999, on the transplanted cells cultures of rat glioma C6 and human neuroblastoma IMR-32 the mitogenic effect of SK (10−11–10−7 M), its stimulation of neuroblastoma cells as well as changes of intracellular contents of DNA, RNA and protein have been demonstrated. The exposition of rat pheochromocytoma PC12 cell culture with 5(10−7–10−6 M) only during 20 min was accompanied by significant changes of the level of ATP and Ca2+-activated intracellular proteolysis.

On organotypical and dissociated cultures of rat neocortex, sensory (spinal) and vegetative ganglia (on the nutrient mediums with deficiency of blood serum proteins), the neurotrophic effect of SK was established. It manifested itself by maintenance of vital activity of cells at deprivation of blood serum proteins, cold stress, damaging effect of anirionic form of ATP, and stimulation of proliferation of glia, Schwann cells and other cells.

The cleavage of bovine fibrinogen in a thin layer of agar gel by washed cells of Pseudomonas aeruginosa hospital strain (grown up on plain agar) was inhibited by 20–100 % after SK additions to the cells on.

Conclusions. The scope of the results creates real preconditions for expansion of the medical application sphere of SK preparations, first of all, for stimulation of tissues regeneration and, perhaps, for treatment of neurological pathology.