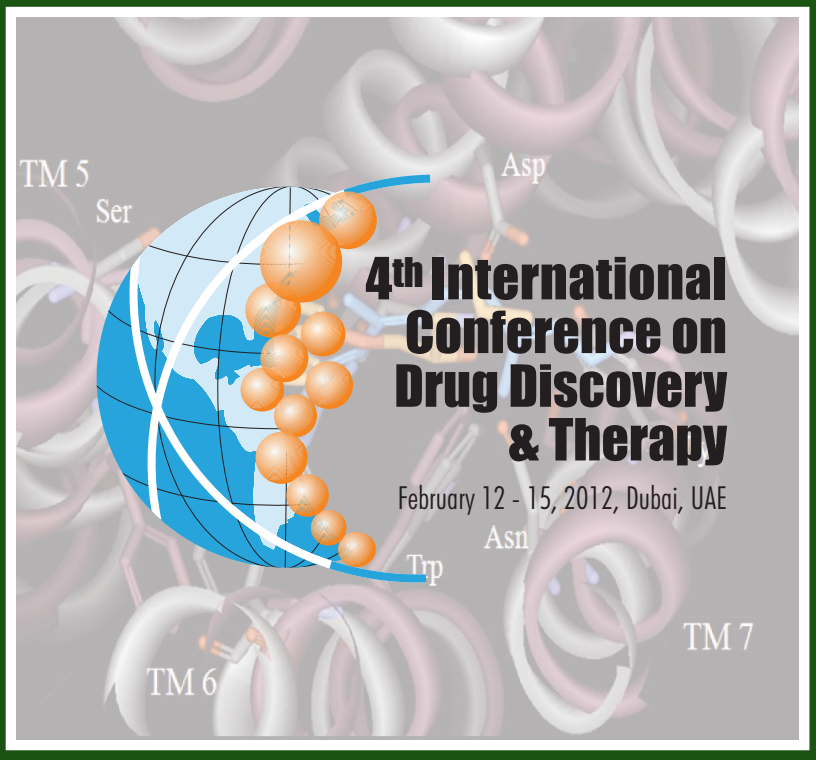
ISSN: 0929-8673

Current Medicinal Chemistry

SCI JOURNAL CITATION REPORTS
Impact Factor: 4.63

ABSTRACTS



The International journal for timely in - depth reviews in Medicinal Chemistry

BENTHAM SCIENCE

4th International Conference on Drug Discovery & Therapy

February 12 - 15, 2012, Dubai, UAE

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4th International Conference

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(Vol. 19, 36 issues) January 2012 - December 2012:
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Aims and Scope

Current Medicinal Chemistry covers all the latest and outstanding developments in medicinal chemistry and rational drug design. Each issue contains a series of timely in-depth reviews and original research articles written by leaders in the field covering a range of the current topics in medicinal chemistry. Current Medicinal Chemistry is an essential journal for every medicinal chemist who wishes to be kept informed and up-to-date with the latest and most important developments.

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POSTERS

WELCOME MESSAGE

It is our pleasure to extend a very warm welcome to the honourable scientists and young researchers participating in the two conferences --- the ***4th International Conference on Drug Discovery & Therapy*** and the ***1st Biotechnology World Congress*** here in Dubai.

This series of conferences has attracted eighteen Nobel Laureates and many other leading scientists to Dubai. The conferences are serving to nurture collaborations with scientists in the region and to establish linkages between scientists in the developing world with those in the advanced Western countries.

Challenges faced by researchers include diseases associated with ageing populations, the spread of transmissible diseases in an interconnected world and the growing threat of resistance to drugs.

We wish to convey our special thanks to **His Excellency Sheikh Nahayan Mabara Al Nahayan**, Minister of Higher Education and Scientific Research, Chancellor, Higher Colleges of Technology for his patronage of these important scientific events. We are also most grateful to all the scientists who have travelled from the four corners of the world to the UAE to participate in these scientific symposia.

We hope that you will find your visit to Dubai intellectually stimulating and socially enjoyable.

PROF. FERID MURAD
(Nobel Laureate)
Co-President

PROF. ATTA-UR-RAHMAN, *FRS*
(UNESCO Science Laureate)
Co-President

PLENARY LECTURES

PL-43

COST EFFECTIVE LEAD DISCOVERY AND DEVELOPMENT- A NEW PARADIGM

M. Iqbal Choudhary and Atta-ur-Rahman

International Center for Chemical and Biological Sciences, (H. E. J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research), University of Karachi, Karachi-75270, Pakistan ; E-mail: hej@cyber.net.pk

As the developing world face food security issues, it is also confronted with drug insecurity challenges. Nation states need to prioritize the agenda for developing safe and effective drugs, against local diseases. The current drug development paradigm is cost, time and labor intensive, in which developing countries cannot afford to invest. There is therefore a need of appropriate, efficient and cost effective strategies for drug development, based on indigenous knowledge resource base, S&T capacity and people-friendly approval process.

Enzyme inhibitors play a significant role in the preservation of human health. The search for new plant based enzyme inhibitory substances has led to the identification of naturally occurring compounds which may be used in the treatment of a variety of ailments. Urease has been an important virulence factor in pathogenic conditions associated with human and animal health. This enzyme is known to be a major source of gastric and peptic ulcers, induced by *Helicobacter pylori*. Urease from *Proteus mirabilis* is also directly involved in urolithiasis, as well as pyelonephritis, hepatic coma, and urinary catheter encrustation. Hence, urease has been an important target of our research, which has led to the discovery of several new classes of enzyme inhibitors.

Diabetes is an old disease which poses new challenges to the human well being. It is characterized by hyperglycemia and associated complications. Among different therapeutic interventions, the discovery of effective α-glucosidase inhibitors and antiglycating agents are considered to be important, based on modern knowledge about the disease at the molecular level. Primary focus of these studies has been to discover lead bioactive molecules by using appropriate conventional and mechanism-based biological screening techniques. As a result, a large number of potent antiglycation agents of natural origin were discovered and structure-activity relationship studies were conducted. Many of these compounds represent new examples of inhibitors of α-glucosidase and glycation processes.

During this presentation, underlying philosophy and approach of our research on cost-effective discovery of lead molecules will be presented.

PL-161

IRX-2 - A NOVEL IMMUNOMODULATOR FOR CANCER

John W. Hadden II

IRX Therapeutics, Inc., 140 West 57th Street, Suite 9B, New York, NY. 10019, USA; E-mail: jhadden@irxtherapeutics.com

Progressive cancer is associated with immune dysfunction and many cancers invoke multiple different immune inhibitory pathways as they progress. An effective immune response to cancer is associated in multivariate analyses as an independent positive prognostic factor. IRX-2 is a biologic comprised of multiple cytokines generated from robust stimulation of donor peripheral blood mononuclear cells with Phytohemagglutinin (PHA). A well-defined manufacturing and release process was established and highly reproducible lots of IRX-2 can be generated. IRX-2 has been shown in multiple pre-clinical models to stimulate dendritic cell activation and generation of antigen-specific T cells. IRX-2 protects T cells from activation-induced cell death and augments the function of Natural Killer cells. IRX-2 reverses HPV-induced inhibition of Langerhans cell activation. We have shown that vaccine induced antigen-specific T cell activation is enhanced when various immunogens presented by peptide, protein, cellular or viral platforms are combined with local injections of IRX-2. In early phase clinical trials, we have shown that the IRX-2 immunomodulatory regimen administered into the area of draining lymph nodes of head and neck cancer patients has only mild local toxicities and induces clear increases in lymphocyte infiltrates into the primary tumors. Both radiologic improvements and improvements in survival are associated with patients who had the highest level of IRX-2 mediated lymphocyte infiltrates. Based on these results IRX Therapeutics is organizing multiple trials to establish the clinical impact of the IRX-2 immunomodulatory treatment including a randomized trial in operable head and neck cancer, a Phase II in HPV-

related cervical intraepithelial neoplasia, and a Phase I/II trial of IRX-2 and a novel WT1 vaccine in patients with hormone refractory prostate cancer.

PL-42

PREVENTIVE VACCINATION AGAINST CANCERS CAUSED BY INFECTIONS

Harald zur Hausen

Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany; E-mail: zurhausen@dkfz.de

At present approximately 21% of the global cancer incidence can be linked to infectious events. By considering only those cancers, close to 1% is due to parasitic infections /(Schist osomiasis and liver flukes), 35% to a bacterial infection (*Helicobacter pylori*) and 64% to viral infections (Hepatitis B and C, high risk human papillomviruses, Human herpesvirus type 8, Epstein-Barr virus, Merkel polyomavirus, Human T-lymphotropic retrovirus, and as indirect carcinogens Human Immunodeficiency viruses). The identification of infectious agents causing human cancers resulted in novel diagnostic procedures and in risk assessment of the infected persons. Even more importantly successful preventive strategies have been developed, permitting the elimination of parasitic infections by chemotherapy, and of *Helicobacter pylori* by antibiotic treatment.

For two wide spread viral infections (Hepatitis B and high risk HPV), jointly causing annually approximately 1 million new cancer cases globally, preventive vaccines became available, which provide long-lasting protection against re-infection with the same agent. These vaccines turned out to be effective in preventing liver cancers caused by Hepatitis B virus and the precursor lesions of cervical cancer caused by the human papillomavirus (HPV) types 16 and 18. Since the average latency period between HPV infection and cancer development usually covers 15-25 years and the vaccine has only been applied since 8-9 years, no statistically significant data have been obtained demonstrating a reduction in the cervical cancer rate. Both vaccines prevent infections with the respective viruses but do not reveal a therapeutic effect. The HPV vaccine should be applied prior to the onset of sexual activity, optimally between 9-17 years of age. There exist a number of reasons not to restrict this vaccine solely to girls. The vaccination of boys should be performed at the same age.

Global acceptance of the HPV vaccine, potential side effects, as well as HPV-DNA based screening procedures will be discussed. World-wide application of both vaccines could reduce cancer incidence in women by 12-15%, in males by 4-5%. Thus, the identification and characterization of cancer-causing viruses permits a novel and very effective approach to prevent specific major human cancers.

PL-41

INTRACELLULAR PROTEOLYSIS, STRUCTURES, MECHANISMS, AND DRUG DESIGN

Robert Huber

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Within cells or subcellular compartments misfolded and/or short-lived regulatory proteins are degraded by protease machines, cage-forming multi-subunit assemblages. Their proteolytic active sites are sequestered within the particles and located on the inner walls. Access of protein substrates is regulated by protein subcomplexes or protein domains which may assist in substrate unfolding dependent or independent of ATP. Five protease machines will be described displaying different subunit structures, oligomeric states, enzymatic mechanisms, and regulatory properties.

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PL-98

FROM SUPRAMOLECULAR CHEMISTRY TOWARDS ADAPTIVE CHEMISTRY BIORGANIC AND DRUG DISCOVERY ASPECTS

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Supramolecular chemistry is actively exploring systems undergoing *self-organization*, i.e. systems capable of spontaneously generating well-defined functional supramolecular architectures by self-assembly from their components, on the basis of the *molecular information* stored in the covalent framework of the components and read out at the supramolecular level through specific interactional algorithms, thus behaving as *programmed chemical systems*.

Supramolecular chemistry is intrinsically a *dynamic chemistry* in view of the lability of the interactions connecting the molecular components of a supramolecular entity and the resulting ability of supramolecular species to exchange their constituents. The same holds for molecular chemistry when the molecular entity contains covalent bonds that may form

and break reversibility, so as to allow a continuous change in constitution by reorganization and exchange of building blocks. These features define a *Constitutional Dynamic Chemistry* (CDC) on both the molecular and supramolecular levels.

CDC introduces a paradigm shift with respect to constitutionally static chemistry. The latter relies on design for the generation of a target entity, whereas CDC takes advantage of dynamic diversity to allow variation and selection. The implementation of selection in chemistry introduces a fundamental change in outlook. Whereas *self-organization by design* strives to achieve full control over the output molecular or supramolecular entity by explicit programming, *self-organization with selection* operates on dynamic constitutional diversity in response to either internal or external factors to achieve *adaptation*.

Applications of this approach in biological systems and to drug discovery will be described.

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PL-1

MEMBRANE PROTEINS: IMPORTANCE, FUNCTIONS, STRUCTURES

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Compared to the membrane lipids membrane proteins are the more active players in biological membranes. They catalyze (i) transmembrane transport, e.g. the specific uptake of nutrients and substrates, the exchange of ions, and the excretion of waste products and extracellular proteins across the membrane (ii) biological energy transfer and energy conservation in photosynthesis and respiration (iii) signal reception, signal transduction across the membrane and amplification (iv) reaction by enzymes with preferentially hydrophobic substrates.

Most drugs available to treat diseases act by inhibiting or activating a membrane protein making membrane protein structure determination extremely interesting for drug design and virtual screening. However, membrane proteins are difficult to study because of material limitations caused by insufficient availability of membrane proteins and their instability. At present the structures of around 300 membrane proteins are known compared to several thousands of water soluble proteins. However, less than 20 structures of human membrane proteins (of about 6000 to 8000) could be determined.

The methods of membrane protein structure determination and several recent successes of the author's lab with membrane proteins of medical interest will be presented.

PL-41

CLIMATE CHANGE: SCIENCE, POLICY AND SOLUTIONS

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Climate change is the most serious environmental challenge facing society in the 21st century. There is little doubt that human activities have modified the composition of the atmosphere: the concentration of carbon dioxide, produced mainly by burning fossil fuels, has increased more than 30% since pre-industrial times. Carbon dioxide is one of several greenhouse gases (GHGs) that trap

energy emitted by the Earth to outer space; other greenhouse gases also affected by human activities are methane and nitrous oxide. The average temperature of the Earth's surface is increasing, and the frequency of extreme weather events such as intense hurricanes, droughts and floods is also increasing. Furthermore, the International Panel on Climate Change has concluded that there is more than 90% probability that this modification in atmospheric composition is the cause of the observed changes in the Earth's climate in recent decades.

The average temperature of the Earth's surface has so far increased by about 0.8 degrees Celsius since the Industrial Revolution. The consensus of informed experts is that the risk of causing dangerous changes to the climate system increases rapidly if the average temperature rises more than two or three degrees Celsius. Society faces an enormous challenge to effectively reduce greenhouse gas emissions to avoid such dangerous interference with the climate system. This goal can only be achieved by taking simultaneously measures such as significantly increasing energy efficiency in the transportation, building, industrial and other sectors, using renewable energy sources such as wind, solar, geothermal and biomass, and even developing and using safer nuclear energy power plants. Fossil fuels such as coal and petroleum can continue to be used beyond a transition period of about one or two decades, but only as long as the emitted carbon dioxide is sequestered and stored in underground reservoirs such as saline domes.

PL-97

DISCOVERY OF NITRIC OXIDE AND CYCLIC GMP CELL SIGNALING AND THEIR ROLE IN DRUG DEVELOPMENT

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The role of nitric oxide in cellular signaling in the past three decades has become one of the most rapidly growing areas in biology. Nitric oxide is a gas and a free radical with an unshared electron that can regulate an ever-growing list of biological processes. Nitric oxide is formed from L-arginine by a family of enzymes called nitric oxide synthases. These enzymes have a complex requirement for a number of cofactors and regulators including NADPH, tetrahydrobioterin, flavins, calmodulin and heme. The enzymes are present in most cells and tissues. In many instances, nitric oxide mediates its biological effects by activating the soluble isoform of guanylyl cyclase and increasing cyclic GMP synthesis from GTP. Cyclic GMP, in turn, can activate cyclic GMP-dependent protein kinase (PKG) and can cause smooth muscles and blood vessels to relax, decrease platelet aggregation, alter neuron function, etc. These effects can decrease blood pressure, increase blood flow to tissues, alter memory and behavior, decrease blood clotting etc. The list of effects of nitric oxide that are independent of cyclic GMP formation is also growing at a rapid rate. For example, nitric oxide can interact with transition metals such as iron, thiol groups, other free radicals, oxygen, superoxide anion, unsaturated fatty acids, and other molecules. Some of these reactions result in the oxidation of nitric oxide to nitrite and nitrate to terminate the effect, while other reactions can lead to altered protein structure function and/or catalytic capacity. These effects probably regulate bacterial infections, inflammation of tissues, tumor growth, and other disorders. These diverse effects of nitric oxide that are cyclic GMP dependent or independent can alter and regulate numerous important physiological events in cell regulation and function. Nitric oxide can function as an intracellular messenger, an antacoid, a paracrine substance, a neurotransmitter, or as a hormone that can be carried to distant sites for effects. Thus, it is a unique molecule with an array of signaling functions. However, with any messenger molecule, there can be too little or too much of the substance, resulting in pathological events. Some of the methods to regulate either nitric oxide formulation metabolism, or function have been in clinical use for more than a century, as with the use of organic nitrates and nitroglycerin in angina pectoris that was initiated in the 1870s. Inhalation of low concentrations of nitric oxide can be beneficial in premature infants with pulmonary hypertension and increase survival rates. Ongoing clinical trials with nitric oxide synthase inhibitors and nitric oxide scavengers are examining the effects of these agents in septic shock, hypotension with dialysis, inflammatory disorders, cancer therapy, etc. Recognition of additional molecular targets in the areas of nitric oxide and cyclic GMP research will continue to promote drug discovery and development programs in this field. Current and future research will undoubtedly expand the clinician's therapeutic armamentarium to manage a number of important diseases by perturbing nitric oxide formation and metabolism. Such promise and expectations have obviously fueled the interests in nitric oxide research for a growing list of potential therapeutic applications. There have been and will continue to be many opportunities from nitric oxide and cyclic GMP march to develop novel and important therapeutic agents. There are presently more than 80,000 publications in the area of nitric oxide research. The lecture will discuss our discovery of the first biological effects of nitric oxide and how the field has evolved since our original

reports in 1977. The possible utility of this signaling pathway to facilitate novel drug development and the creation of numerous projects in the Pharmaceutical and biotechnology industrials will also be discussed.

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PL-160

PLx PHARMA - DEVELOPING A GI SAFER ASPIRIN

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PLx Pharma Inc. is developing a GI safer formulation of aspirin (PL 2200) which achieved a 71% reduction of risk for ulcers in a recent clinical trial comparing it to regular aspirin. PLx's GI protective formulation utilizes a naturally occurring refined soy derivative, phosphatidylcholine (PC), in pre-association with aspirin to maintain aspirin's therapeutic effectiveness while substantially reducing undesirable GI bleeding and ulceration. This product has the potential to positively impact patients and overall healthcare costs by enabling aspirin to be used in a broader population for prevention of the two leading causes of death, cardiovascular disease and cancer. PLx is preparing to file a New Drug Application for PL 2200 in the USA in the first quarter of 2012.

Clinical Results. In a six site endoscopy trial of 200 patients over 50 years of age which compared PLx's lead product, PL 2200 Aspirin 325 mg, to 325 mg immediate release regular aspirin, PL 2200 achieved a 71.0% reduction of risk for ulcers ($p = 0.0069$). PL 2200 has also successfully completed bioequivalence trials which demonstrated bioequivalence to 325 mg immediate release aspirin while maintaining full antiplatelet activity.

PL 2200 Market Opportunity. Studies show that 10% of low dose aspirin users will develop ulcers and 2.5% will develop potentially deadly bleeds, with 30% of NSAID-induced deaths caused by low dose aspirin use. In the US alone, 43 million Americans take aspirin for prevention of heart attack and stroke with most taking 81 mg. For many of these 325 mg aspirin is the appropriate antiplatelet dose but they cannot tolerate a regular 325 mg dose. A similar number of patients with cardiovascular disease do not take any aspirin as they cannot tolerate it. PLx will address these large and growing markets in the US and globally. This will expand the use of a GI safer 325 mg dose aspirin to diabetics and obese whom are at high risk for cardiovascular disease and need a GI safe full antiplatelet dose of aspirin. It will also allow arthritis sufferers using other NSAIDs and aspirin together, which places them at high risk for GI problems, to have a safer aspirin for cardio benefit.

PL2200 Aspirin will provide a safe alternative to anti-secretory drugs, which are limited by drug interactions and a growing risk of potential side effects, including an elevated risk of fractures and infections.

There have been a number of retrospective studies suggesting that chronic aspirin use may significantly reduce the risk of several cancers. A recent prospective study of gastrointestinal cancers provides confirming evidence of aspirin's potential to reduce the risk of GI cancer. While additional prospective studies need to be undertaken, a GI safer aspirin that enables chronic use of 325 mg daily or BID by reducing aspirin's GI toxicity may have a profound impact on cancer rates and patient outcomes.

Additional clinical trials are in preparation that aim to demonstrate that PL 2200 is faster acting than enteric coated aspirin and a more reliable antiplatelet agent.

PLx's NSAID-PC Platform. In addition to PL 2200, PLx's product pipeline includes GI protective formulations of ibuprofen, as well as other currently marketed NSAIDs. NSAIDs induce GI toxicity in part by disrupting the naturally occurring PC barrier to acid that is found in the stomach lining. By non-covalently pre-associating an NSAID with PC, the NSAID becomes more lipophilic allowing it to move through the stomach's barrier to acid with minimal disruption. This NSAID-PC complex more safely delivers an equivalent therapeutic dose of the NSAID into systemic circulation by

mitigating the interaction between the NSAID and the naturally occurring PC. As a result, the gastric protective barrier can remain intact and repel acid without altering the acid level in the stomach, thus avoiding the side effects of the current standard of care, chronic acid suppression.

SPECIAL INVITED LECTURES

SIL-61

Track: *Academic CRO/Industrial collaborations in drug discovery*

ENABLING NEW PHARMA: DRUG REPURPOSING EFFORTS GO MAINSTREAM

Rathnam Chaguturu

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Finding new uses or indications for existing medications has gained unprecedented momentum in recent years. NIH's new venture in setting up Drug Discovery Institute is primarily to accelerate the drug repositioning efforts. There are over 50 companies with unique capabilities to support drug repositioning efforts. The approach is now systematic, and has become the chosen route for a number of biotechs and small pharmas. These 'drugs' include not only the FDA approved drugs, but also the drugs that have been withdrawn, and those that have gone through clinical trials but not marketed. Since these drugs have a significant 'safety' profile and possibly conquered the 'valley of death', the cost of taking these drugs in to the market is low, and faster. The questions remain as to who owns the intellectual property, and what prevents a physician prescribing the drug for off-label uses. The talk will analyze the drug repositioning strategies, assess opportunities and business models, highlight major challenges, explore intellectual property barriers, etc.

SIL-44

Track: *Drug Metabolism*

MECHANISM OF DRUG TOXICITY AND RELEVANCE TO PHARMACEUTICAL DEVELOPMENT

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Drug discovery and development is a time consuming and costly process. On average, it takes approximately ten to twelve years and of the order of $1.3 billion to bring a new drug to market and attrition of the process is very high. It has been estimated that for every 5000 new chemical entities evaluated in a discovery program, only one is approved for market. Even after a drug is marketed there is a possibility that the drug is either withdrawn from the market or acquires a warning label (black box) due to adverse drug reactions. Therefore, efforts are being made to reduce the attrition of drug candidates and to select the best candidate on the basis of optimal PK/PD response and desirable metabolic and safety profiles in order to bring safer and efficacious compounds to the market. One of the reasons of drug toxicity is bioactivation. The metabolic activation of drugs results in the formation of reactive metabolites, which covalently bind to proteins to form the drug-protein adducts termed "foreign" proteins, which lead to an immune response and toxicity. Efforts are routinely made to detect and identify these reactive metabolites and to guide the medicinal chemists to synthesize the molecules with no or low potential of bioactivation. This presentation will describe the approaches for the detection of and identification of reactive intermediates; provide guidance for discovery scientists in applying the information to minimize the bioactivation potential and risk mitigation strategies in dealing with reactive metabolites issues at candidate nomination stage.

INVITED LECTURES

IL-127

Track: *Inflammation & Immunology*

PROPHYLAXIS OF THE AUTOIMMUNE DISEASES

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Microbial triggers. It is now clear that autoimmune diseases are caused by faulty immune responses to specific microbial infections, Alan Ebringer leading the search for these triggers.

Forbidden Clones. Following Niels Jerne's epochal realization that antibodies are not built on a template of antigen, but are pre-formed in myriad diversity, awaiting selection by an antigen that fits, Macfarlane Burnet realized that it is not antibodies that are selected but the cells that make them, and that these are the lymphocytes. Burnet proposed that each lymphocyte is covered in multiple copies of a single antigen receptor to form an immunological clone, with millions of cells in a clone and millions of different clones in a person. This is his Clonal Selection Theory of the immune response. Furthermore, he realised that multiplying lymphocyte will mutate, and if the mutation involved is in a V (variable region) gene, a new clone will be formed. Hence, his Forbidden Clone Theory, postulating that unlucky mutations in V genes cause the autoimmune diseases. This theory has been amply confirmed in detailed studies of Graves' disease, which is caused by B lymphocyte forbidden clones, whereas T lymphocyte forbidden clones cause Type1 diabetes.

H Gene and V Gene risk factors. Autoimmune diseases run in families, being caused by multiple codominant genes with incomplete penetrance. The H Gene Theory of Adams and Knight explains all the features of the genetics by postulating that Histocompatibility antigens, major, minor and H-Y, dictate the immune repertoire by deleting nascent complementary clones, and so altering the risk of occurrence of the various autoimmune diseases.

Specific immunotherapy. Immune ablation and autologous bone marrow cell reconstitution: Because unlucky somatic mutations in multiplying lymphocytes cause the autoimmune diseases, they are unlikely to recur in regenerating immune repertoires following immune ablation and autologous bone marrow cell reconstitution, as shown by Englert *et al.* with cases of lethally severe systemic scleroderma.

Manufacture and use of cytotoxic autoantigen complexes: After isolation of its autoantigen, an autoimmune disease should be curable by selective destruction of its forbidden clone by making and using a cytotoxic autoantigen complex.

Prophylaxis. This will be possible by identifying and vaccinating against microbial triggers of the various autoimmune diseases. Johannes Salk's anterior poliomyelitis vaccine has prevented the leg paralyses that must have been a rare autoimmune complication of the virtually universal infections with the virus occurring in poliomyelitis epidemics.

Reference:

Adams DD, Knight JG, Ebringer A. Autoimmune diseases: Solution of the environmental, immunological and genetic components with principles for immunotherapy and transplanation. *Autoimmunity Reviews* 2010; 9: 525-30.

IL-168

Track: *Inflammation & Immunology*

THE ESSENTIAL ROLE OF THE HISTOCOMPATIBITY SYSTEM FOR VIRUS DEFENCE AND HOW TRANSPLANT SURGEONS CAN XENOGRAFT WITHOUT REJECTION

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Oncologists wishing to study tumours by transplanting them from their source to another laboratory animal, found that the tumours were rejected. With transplantation of skin in people, Gibson and Medawar observed that on second occasions grafts were rejected faster, concluding that an immunological process is involved. The first successful kidney transplant was performed between identical twins, demonstrating the genetic basis of rejection. To emulate identical twins for acceptance of foreign grafts, oncologists used brother-sister mating of rodents to produce inbred strains.

This led to discovery of the histocompatibility system governed by a major genetic complex named the major histocompatibility complex (MHC). These genes code for surface antigens on all nucleated cells. The MHC is essential

for host survival of virus infections. Zinkernagel and Doherty found that a cell infected by a virus extrudes a viral peptide on to its surface histocompatibility antigens where it can be attacked by a complementary cytotoxic T cell clone, if one exists. Fenner and White discovered that the replication time for influenza virus is 10 hours, after which 1,000 progeny are released, each able to infect another cell. The defending cytotoxic T cells replicate in 18 hours, producing only two new cells. Adams has done the relevant sums, which show that in the race between virus and cytotoxic T cell, the virus/T cell ratio becomes 10^{14}/1 in 3 days, so the virus wins and the patient dies. This explains why foreign grafts, which are mistaken for virus-infected host cells by the immunity system, are attacked so strongly. Kaplan discovered that animals can be made haematological chimeras by total lymphoid irradiation followed by inoculation with allogeneic bone marrow after which they will accept skin and organ grafts from the donor of the bone marrow. Sykes found reconstitution with recipient bone marrow as well as donor is an improvement. Adams has reported that the scene is set for use of untreated pigs as ideal organ donors for man.

Reference:

Adams DD. Why the histocompatibility system exists and how transplant surgeons can xenograft without rejection. *Q.J. Med* 2011; 104: 767-769.

IL-63

Track: *Hot Topics in Medicinal Chemistry*

AN APPRAISAL FOR THE DEVELOPMENT OF THERAPEUTICS FOR ALZHEIMER'S DISEASE: DESIGN, SYNTHESIS AND QUALITATIVE STRUCTURE ACTIVITY EVALUATIONS OF NOVEL β-SECRETASE INHIBITORS

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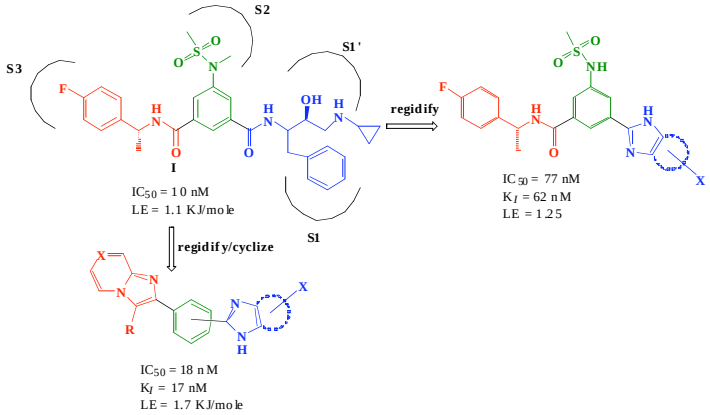
We have identified highly selective small molecules substituted imidazopyridine armed with benzimidazol and/or aryl imidazole as potent β-secretase inhibitors. The most effective and selective analogs demonstrated low nano-molar potency for the BACE1 enzyme as measured by FRET and a cell-based (ELISA) assays and exhibited comparable affinity (K_I) and high ligand efficiency (LE). In addition, these motifs were highly selective (>200) against the structurally related aspartyl protease BACE2. Our design strategy followed a traditional SAR approach and was supported by molecular modeling studies based on the previously reported hydroxyethylene transition state inhibitor derived from isophthalic acid I. In this regard, we took advantage of the isosteres, imidazole and amide groups armed at the isophthalic acid derivatives of type I, to build potent ligands with nano-molar activities against BACE1 that exhibited high selectivity against BACE2. Of the most potent compounds, 34, displayed an IC_{50} value for BACE1 of 18 nM, and exhibited cellular activity with an EC50 value of 37 nM in the cell-based ELISA assay, high affinity (K_I = 17 nM) and ligand efficiency (LE = 1.7 kJ/mol). Compound 34 was found to be 204-fold more selective for BACE1 compared to the closely related aspartyl protease BACE2 [1].



Reference:

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IL-70

Track: *Cardiovascular Drug Discovery & Therapy*

NEW NO-RELEASING ANTIISCHEMIC AGENTS: USING PYRIDOXINE AS A TEMPLATE

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Addressing cardiovascular pathologies including ischemic injury hybrid molecules have been synthesized- these hybrid molecules possess nitric oxide (NO) releasing properties. Nitric oxide (NO) releasing donors have now been added to known therapeutic agents such as aspirin, naproxen and many others to produce the desired vasodilating effect. Administration of nitric oxide (NO) donors during ischemia and reperfusion protects from myocardial injury. However, whether administration of an NO donor during a brief period prior to ischemia protects the myocardium and the endothelium against ischemia-reperfusion injury in vivo is unknown. To study this possibility pyridoxine based nitric oxide donors have been synthesized. The present investigation is based on our previously reported phosphonates1; to further elaborate on this program, we have prepared α-fluoro pyridoxine 5´-phosphonates containing oxynitrite/nitro group at the phenolic nitrooxymethyl of α-fluoro pyridoxine 5´.

IL-181

Track: *Regenerative Medicine*

ARTIFICIAL LIGAMENTS RECONSTRUCTION: RECENT DEVELOPMENTS

Djedjiga Benouioua-Ait Aouit

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Background: Although significant progress has been made toward understanding the anatomy, composition, biomechanics and healing of ligaments, no graft or prosthesis ideally suited to substitute natural ligaments so far. Natural ligaments are capable of withstanding high stresses and during the 1970s and 80s, various synthetic materials were designed to act as a permanent ligament replacement devices. In fact, these artificial ligaments did not possess the same biomechanical properties of the native structure and were known to fatigue, stretch, and/or particulate over time. The major mechanisms of artificial ligament failure included very poor biomechanics of resisting flexion and torsion load (Hagemeister et al 2002). These factors resulted in fatigue rupture or elongation of the artificial ligament and severe synovitis of the knee caused by wearing particles of artificial ligament fibers (Yahia 1997). The super-helical organization of fibrils in canine and human anterior cruciate ligaments (ACL) was recently confirmed by Vidal Bde et al (2009). This natural ligamentous organization inspired the development of a novel artificial ligament scaffold—the Ligament Advanced Reinforcement System (LARS; Surgical Implants and Devices, Arc-sur-Tille, France).

In this work, we evaluated the biomechanical properties of this new generation of artificial ligament. The biomechanical experiments such as the flexion-extension test, the laxity test and the destructive test were performed on 28 ewe's stifles three months after their implantation. In order to identify other reasons of the rupture of the artificial ligament, the 3D position of the screw relative to the anatomical landmarks is quantified to both ruptured and unruptured ligaments and compared using Student test.

Material and Method: The proposed Ligament Advancement Reinforcement System (LARS) consists of 44 fibers of PETG (polyethylene terephtalate glycol). It could be grafted with a bioactive polymer that can control the cellular responses. So, fourteen ewes were implanted. One knee received the LARS ligament in place of the native ligament and the contralateral knee allowed to make comparisons. The 14 ewes were divided into two groups of 7 ewes: one group received the artificial ligament grafted with a bioactive polymer and the other group received the artificial ligament without grafting. To measure the angles of the screw according to the anatomical landmarks, EOS images of the implanted stifles are acquired.

Results: The biomechanics curves obtained on the stifles implanted with the grafted ligaments have tendency to be superimposed with those obtained on the stifles with native ligaments. However, the curves obtained on the stifles implanted with non grafted ligaments with bioactive polymer showed a significant difference. The obtained average

angles of the tibial screw in the projection plane XOY showed that the null hypothesis was rejected for the student test parameter t=3.45 with the confidence level p-value = 0.005.

Conclusion: The native ligaments give better results of relaxation, stiffness and failure load than the artificial ligament. However, the results between the grafted artificial ligaments and non grafted ones are encouraging for the grafting. The average angles measured on the screw according to the tibial landmarks of the ruptured ligament samples are significantly different from that of the unruptured ligament samples.

This study was supported by the Laboratory of BioMechanics, Ecole Nationale Superieure d'Arts et Metiers-Paris and Ecole Nationale Vétérinaire d'Alfort-Paris.

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IL-16

Track: *CNS Drug Discovery & Therapy*

THE PACAP RECEPTOR: A NOVEL TARGET FOR MIGRAINE TREATMENT

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Fast tracking of new drugs for migraine has faced the major challenge of poorly-predictive preclinical models. Indeed, novel, migraine-specific preventive drugs have not been developed in decades. Furthermore, the acute therapy armamentarium remains suboptimal, with no new chemical entities since the triptans. On the other hand, human experimental studies are surfacing as a powerful tool in targeted migraine therapy. Over 20 years of research in human models have led us to develop a research paradigm for the identification of novel migraine drug targets, which combines human provocation studies and preclinical models. Recent human experiments have shown that pituitary adenylate cyclase-activating polypeptide (PACAP-38) infusion induces marked dilatation of extracerebral vessels and migraine attacks in migraine patients. PACAP is present in sensory trigeminal neurons and may modulate nociception at different levels of the nervous system. Furthermore, PACAP selectively activates the PAC1 receptor, which suggests a possible signaling pathway implicated in migraine. I will review the current evidence supporting the involvement of PACAP in migraine pathophysiology and the PAC1 receptor as a possible novel target for migraine treatment.

IL-101

Track: *Recent Advances in Patient Treatment and Care*

BIOMARKER DISCOVERY FOR FABRY DISEASE USING A MASS SPECTROMETRY APPROACH

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Fabry disease is an X-linked, multisystemic lysosomal storage disorder characterized by the accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb_3) in biological fluids, vascular endothelium, heart, and kidneys. Treatment by enzyme replacement therapy is provided to both males and females depending on the severity of symptoms. We devised rapid and efficient tandem mass spectrometry methodologies using a Waters Quattro micro-HPLC Alliance system to quantify two urinary Fabry disease biomarkers: Gb_3 (4 min-assay) and globotriaosylsphingosine (lyso-Gb_3) (6 min-assay) analyses normalized to creatinine (creat). We characterized urinary lyso-Gb_3 by time-of-flight mass spectrometry on a Waters Synapt UPLC-QTOF MS (Ultra-Performance Liquid Chromatography-Time-of-flight mass spectrometry) system, since it was previously only detected in blood. Validation

of both methodologies for clinical use gave good coefficients of variation for intra-day and inter-day assays (<13%). Normal values were established for adult Gb_3 (<25 ug/mmol creat) and lyso-Gb_3 (none found in the control cohort). The objectives of this study was to identify and characterize novel disease-specific biomarkers in patients affected with Fabry disease that reliably reflect disease progression and severity, and thereby facilitate the evaluation of new therapies by improved monitoring of the response to treatment. We employed a time-of-flight mass spectrometry metabolomic approach. Urine and plasma samples from untreated and treated Fabry patients were evaluated and compared to age-matched controls. All samples were analyzed on a UPLC-QTOF MS Synapt system. Results processed with MarkerLynx (Waters) were downloaded in EZInfo (Umetrics) for multivariate data analysis such as principal component analysis (PCA) and orthogonal partial least square-discriminant analysis (OPLS-DA). We detected specific analogs in both biological fluids under study: in plasma, we found three novel lyso-Gb_3 analogs at *m/z* 802; *m/z* 804; and *m/z* 820 and four other urinary analogs of lyso-Gb_3 at *m/z* 758*; m/z* 774; *m/z* 800; and *m/z* 836. A *m/z* 784 analog was found in both biological fluids. Area counts for Fabry analogs were compared to controls. We found that some urinary analogs presented higher area counts than lyso-Gb_3. We confirmed that all analogs are lyso-Gb_3 sphingosine moiety modifications. Correlations between the presence and amounts of various disease-specific analogs and specific indices of clinical severity are in progress. To our knowledge, this metabolomic study using time-of-flight mass spectrometry is the first to demonstrate the presence of analogs of lyso-Gb_3 potentially quantifiable by tandem mass spectrometry. The next step will be to synthesize efficient standards to accurately measure the amounts of these biomarkers in biological fluids. This will lead to the evaluation of correlations to determine disease-severity and progression in Fabry disease patients.

IL-62

Track: *Drug Metabolism*

MOLECULAR DETERMINANTS OF THE INTERACTION OF ANTHRACYCLINES WITH CHROMATIN

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Anthracyclines are a powerful group of antitumor drugs used in the treatment of many different cancers. However, their molecular mechanisms of action are still poorly understood [1]. One of such basic mechanisms involves their intercalation between the bases of DNA which takes place within the context of chromatin. Over the years my lab has extensively characterized the interaction of daunomycin with chromatin using different biochemical and biophysical approaches including analytical ultracentrifugation, circular dichroism and fluorescence. We have found that this cancer antibiotic has a strong binding preference for the histone-depleted linker DNA regions connecting adjacent nucleosomes in the chromatin fiber. This, results in an unfolding of the chromatin that is followed by massive aggregation presumably due to histone-DNA interfiber interactions [2]. We have also provided novel evidence that daunomycin can preferentially bind not only to linker DNA but also to the linker histones in the native chromatin setting without any noticeable binding to the core histones in the nucleosome. Such restrictive binding most likely reflects the constraints imposed by the structural organization of chromatin on its interactions with the drug as daunomycin is able to bind with the same binding affinity to both linker and core histones when free in solution [3].

More recently, we have produced data using chromatin templates with high levels of histone acetylation to mimic the structural and compositional features of transcriptionally active chromatin. The results thus obtained have shown that histone acetylation enhances nucleosome dissociation and decreases the extent of aggregation of the chromatin fiber in a way that is modulated by the presence of linker histones. Histone acetylation increases the binding affinity of daunomycin by chromatin, suggesting that within the cell, this drug preferentially interacts with transcriptionally active chromatin domains [4].

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IL-179

Track: *"Anti-Cancer Drug Discovery & Therapy"*

THE HIGH AFFINITY OUABAIN RECEPTORS' DYSFUNCTION AS A PRIMARY MECHANISM FOR CANCEREGENESIS

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The overhydration is one of the essential diagnostic parameters for cancer cells and serves as a messenger for activation of abnormal cell proliferation and depressing apoptosis. However the nature of the mechanism of dysfunction which brings to over-hydration in cancer cell is not clear yet. In our previous work was shown that the woman breast cancer tissues' hydration was more sensitive to nanomolar ouabain concentrations than normal one. Therefore, to reveal the individual role and mechanism of high affinity ouabain receptors (α3 catalytic subunits of Na/K-ATPase) in regulation of cell hydration of different organs in healthy and having carcinoma-180 tumor mice the dose-dependent oubain effect on45Ca uptake and efflux, intracellular cGMP content, tissue hydration and 3H-timidin involvement in DNA proliferation, and 3H-ouabain binding with membrane receptors were studied. In cancer cells the depression of ouabain affinity of α3 receptors, which was accompanied by the decrease of intracellular cGMP formation, inhibition of 45Ca efflux, dysfunction of metabolic control of cell hydration and increase of the 3H-timidin involvement in DNA proliferation were observed. The depression of α3 catalytic subunits of Na/K-ATPase is suggested as a primary mechanism for dysfunction of the Na/K pump-dependent cell volume control, leading to cell over-hydration and abnormal activation of proliferation.

IL-50

Track: *Drug Metabolism*

ANTICIPATED KEY CHANGES IN THE FDA'S DRAFT DDI GUIDANCE, AND EMERGING DIFFERENCES

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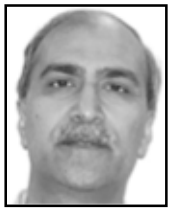

The FDA and the Consortium of companies on Innovation and Quality met in June 2011 in Washington, D.C. to discuss the upcoming changes in the FDA's new draft guidance on DDI predictions. The guidance is anticipated to be issued shortly. It was also attended by the EU regulators and industry experts describing new developments and challenges in accurate DDI predictions. This presentation will highlight key changes anticipated in the way the DDIs could be predicted, with a major emphasis on the use of static and dynamic models for CYP-based DDI predictions. Inclusion of guidelines on transporter based DDI assessments was also included, generally in line with the International Transporter Consortium's White paper that was issued in 2010. Since those discussions some research evidence has emerged that would impact the guidances. The presentation will bring up and discuss those key aspects and challenges.

IL-194

ANTI-LEUKEMIC RESPONSE OF TOLFENAMIC ACID

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Specificity protein (Sp) transcription factors mediate the expression of several genes associated with

cancer. Since tolfenamic acid (TA), targets Sp transcription factors, we have investigated the antileukemic efficacy of TA in pre-clinical experiments. The anti-proliferative response of TA was determined using Jurkat, Nalm6, Molt-4, THP1, NB4, KG1 cells. Selected cell lines, Jurkat, and Nalm6 were used for further experiments to test the effect of TA on apoptosis, cell cycle, and the expression of Sp proteins, survivin and cleaved poly ADP-ribose polymerase (c-PARP). TA showed a steady and consistent decrease in cell viability of all tested cells. TA resulted in an increase in the apoptotic fraction (annexin V positive) of cells, accompanied with an increase in caspase 3/7 activity and up-regulated the cleavage of PARP. TA caused significant decrease in the expression of Sp1, Sp3 and survivin, and blocked the cell cycle progression affecting early phases of cell cycle. These results indicate that TA effectively inhibits human leukemia cell growth potentially through causing cell cycle arrest, suppressing Sp1, Sp3 and surviving expression, and inducing apoptosis. This study strongly supports the development of TA as an effective anti-leukemic agent. Further research on the use of TA in leukemia is currently under investigation.

Financial support: MD Anderson Cancer Center Orlando and TJ Martell Foundation.

IL-180

Track: *Anti-Infective*

DISCOVERY AND DEVELOPMENT OF THE HCV-PROTEASE INHIBITOR: INCIVEK

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Incivek is a newly approved treatment for chronic genotype 1 hepatitis C infection (HCV). The discovery, from protein crystal structure, design, pharmacokinetics, to clinical development data of this HCV-protease inhibitor will be presented.

IL-117

Track: *Drug Delivery & Targeting*

THE EFFICACY OF EXPAREL™, A MULTIVESICULAR LIPOSOMAL EXTENDED RELEASE BUPIVACAINE

Sergio Bergese

The Ohio State University, Columbus, OH, USA

Introduction: EXPAREL is a recently FDA-approved long-acting local analgesic. A well-tolerated safety profile has been demonstrated. Efficacy in wound infiltration studies has also been demonstrated for three days in multiple trials, which are summarized here.

Methods: The EXPAREL wound infiltration program encompassed multiple dosing comparisons throughout ten clinical trials; nine of these were randomized, parallel-group clinical trials, seven of which had a bupivacaine control and two of which had a placebo control. A total of 823 patients were exposed to EXPAREL at doses from 67 mg to 532 mg in both soft tissue and orthopedic models across five different surgical procedures: hemorrhoidectomy, bunionectomy, breast augmentation, total knee arthroplasty, and inguinal hernia repair. In those studies, 446 control patients received bupivacaine (dose: 75 mg to 200 mg) and 190 received placebo. Efficacy was assessed by multiple methods, with a program-wide endpoint of the area under the curve of the numeric rating scale for pain at rest through 72 hours applied.

Results: The pivotal trials met their primary endpoint the numeric rating scale scores for pain at rest combined to generate the area under the curve through 72 hours (hemorrhoidectomy) and through 24 hours (bunionectomy) with $P<0.0001$ and $P=0.0005$, respectively. When a program-wide endpoint of the area under the curve of the numeric rating scale score for pain at rest from 0 through 72 hours was applied, statistical significance was again achieved in both the soft tissue and orthopedic models favoring EXPAREL over the control arm, with greater differences at 72 hours compared to 24 hours.

Conclusion: EXPAREL, as demonstrated by the body of evidence presented, has efficacy as a long-acting local analgesic for three days and may therefore be useful in the reduction of postsurgical pain.

Summary: EXPAREL is a long-acting local analgesic with efficacy for three days and a well-tolerated safety profile; this may have utility as a foundational element in a multimodal regimen for the reduction of postsurgical pain

INTRODUCTION

- Local anesthetics/analgesics are commonly used as part of a multimodal therapy for pain management
- Bupivacaine, which has been shown to reduce postsurgical pain when used via infiltration, is the longest acting local anesthetic/analgesic, but is limited to a duration of 7 hours or less[1]
- EXPAREL, a liposome injection of bupivacaine, is a long-acting analgesic indicated for administration into the surgical site to produce postsurgical analgesia; a well-established safety profile has been demonstrated
- In multiple wound infiltration studies, efficacy has been demonstrated for three days
- EXPAREL utilizes the multivesicular DepoFoam® product delivery platform to encapsulate medications without altering their molecular structure and release them over a desired period of time (Figure 1)

IMAGE OF DEPOFOAM

Fig. (1). DepoFoam Multivesicular Liposome Technology

- DepoFoam utilizes membrane components that are based on natural and well tolerated sources, that are cleared by normal metabolic pathways
- DepoFoam is <3% lipid, biodegradable, and biocompatible
- The DepoFoam technology is currently used in three commercially available products

METHODS

- A total of 10 wound infiltration studies were conducted in both soft tissue and orthopedic surgical procedures, during which a total of 823 patients were exposed to EXPAREL at doses ranging from 67 mg to 532 mg (Table 1)
  - 446 control patients received bupivacaine at doses ranging from 75 mg to 200 mg
  - 190 patients received placebo
- Two of the 10 wound infiltration studies were pivotal trials. These included a soft tissue surgical model (hemorrhoidectomy) and an orthopedic surgical model (bunionectomy)
- The program-wide efficacy endpoint for all wound infiltration studies was the cumulative pain scores through 72 hours, defined as the area under the curve (AUC) of the numeric rating scale (NRS) scores for pain at rest
  - Additional methods were used to assess efficacy on a trial-by-trial basis, including opioid consumption and patient satisfaction

Table 1. All Wound Infiltration Studies

Note: add doses will be updated

Table 1. All Wound Infiltration Studies

Surgery	Control (Pivotal or Supportive)	Type of Surgery	EXPAREL Doses (mg)	Bupivacaine HCl Dose (mg)
General	Pivotal (placebo)	Hemorrhoidectomy	266	N/A
	Active (supportive)	Hemorrhoidectomy	266	100
		Hemorrhoidectomy	67, 200, 266	75
	Active (supportive)	Hernia Repair	93, 160, 236	105
		Hernia Repair	155, 200, 266, 310	100
Orthopedic	Pivotal (placebo)	Bunionectomy	106	N/A
	Active (supportive)	Total Knee Arthroplasty	532	200
		Total Knee Arthroplasty	133, 266, 399, 532	150
Reconstructive	Active (supportive)	Breast Augmentation	532	200
		Breast Augmentation	133, 266	75

RESULTS

Efficacy Demonstrated in Soft Tissue and Orthopedic Pivotal Trials

- Both pivotal trials were multicenter, randomized, double-blind, placebo-controlled studies and met their respective primary efficacy endpoint of pain reduction as assessed by cumulative pain scores (AUC of NRS)
- Each trial met the prespecified primary endpoint with a statistically significant decrease in cumulative pain score through 72 for hemorrhoidectomy (P<0.0001) and through 24 hours for bunionectomy (P=0.0005), respectively (Figures 2 and 3)

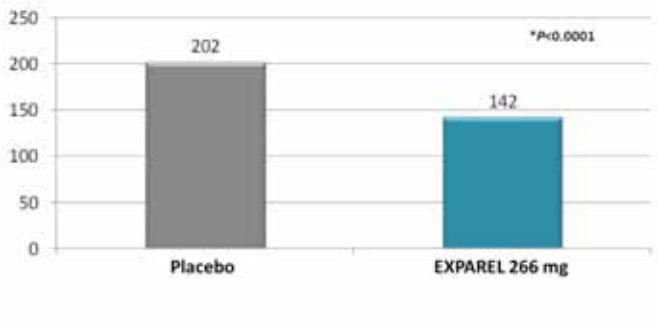

Fig. (2). Reduction in Cumulative Pain Score in Hemorrhoidectomy Pivotal Trial.

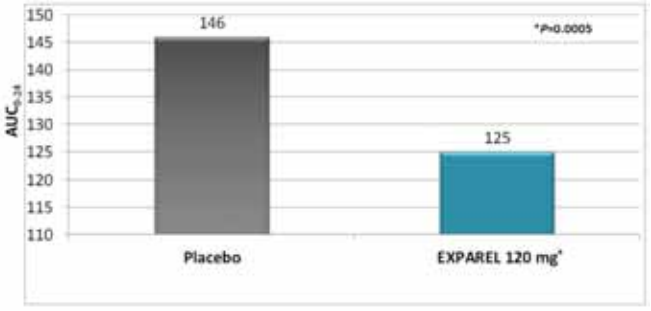

Fig. (3). Reduction in Cumulative Pain Score in Bunionectomy Pivotal Trial

Efficacy Demonstrated Across All Wound Infiltration Trials

- When the efficacy endpoint of cumulative pain score from 0 to 72 hours was applied across the wound infiltration trials, statistical significance was achieved in both the soft tissue and orthopedic models favoring EXPAREL over the control arm
- Greater differences favoring EXPAREL were seen at 72 hours compared to 24 hours
- Figures 4 and 5 present the differences in cumulative pain scores (AUC for pain at rest) from 0 to 24 hours and from 0 to 72 hours between the EXPAREL and controls groups for every dose in each of the parallel-group wound infiltration studies (19 doses in 9 studies)
- The circles represent the difference in means and the bars represent the 95% confidence intervals for the difference in mean
- The red perpendicular line represents the zero line, which indicates no difference in means between EXPAREL and control
    - If a confidence interval does not cross the zero line, there is a statistically significant difference between EXPAREL and the control; otherwise, while a trend may exist, it does not reach a level of statistical significance
- There were 17 treatment arms comparing EXPAREL with bupivacaine HCl in active-controlled trials

- In 5 groups the analysis of the cumulative pain score through 72 hours demonstrated that EXPAREL was statistically superior to bupivacaine HCl ($P<0.05$)
- In 8 groups there was a trend toward favoring EXPAREL, 3 groups failed to show a difference, and in only 1 group there was a trend favoring bupivacaine HCl

- The graphs support a body of evidence that favors EXPAREL through 24 hours and then favors EXPAREL more strongly through 72 hours
- In Phase 2 dose-ranging studies including sub-therapeutic doses, the results still favor EXPAREL and a dose response trend is often noted
- Percentage of patients who exhibited at least 1 treatment-emergent adverse event for EXPAREL, bupivacaine, and placebo: 62%, 75%, 43%, respectively
- One death each occurred in the EXPAREL and bupivacaine groups—both were deemed by the investigator to be unrelated to the study drug
- The most common treatment-emergent adverse events reported with EXPAREL were nausea, constipation, and vomiting

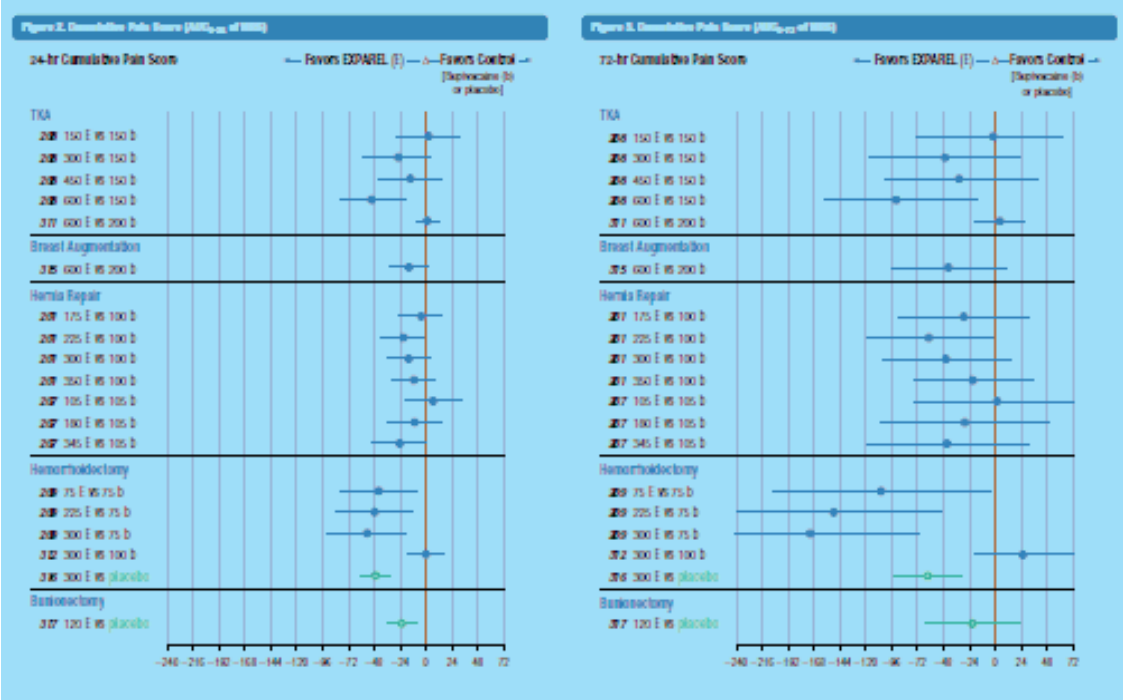

Fig. (4). Cumulative Pain Score ($AUC_{0\text{-}24}$ of NRS-R).

Fig. (5). Cumulative Pain Score ($AUC_{0\text{-}72}$ of NRS-R).

Note all of the EXPAREL dosing will be updated

CONCLUSIONS

- Statistical significance or a trend favoring EXPAREL over bupivacaine HCl was demonstrated in 13 of the 17 active-controlled trials (76% of the trials conducted); given the fact that 50% of pain trials with known active ingredients fail to show a separation of efficacy from placebo,[2] these results further substantiate the utility of EXPAREL for postsurgical analgesia across multiple surgical models
- EXPAREL, a long-acting local analgesic indicated for administration into the surgical site to produce postsurgical analgesia, has demonstrated efficacy for three days across a variety of surgical procedures when administered via wound infiltration

- Wound infiltration as part of a non-opioid, multimodal analgesic regimen is recommended to successfully control postsurgical pain[3]; therefore, extending the duration of action of a local analgesic may reduce the reliance on supplemental medications to treat breakthrough pain
- EXPAREL has the potential to become a foundational element to manage postsurgical pain in soft tissue and orthopedic surgeries

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IL-199

Track: *Anti-Cancer Drug Discovery & Therapy*

INHIBITORS OF THE MULTIDRUG BCRP TRANSPORTER: DESIGN, SYNTHESIS, *IN VITRO* AND *IN VIVO* VALIDATION

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The ability of cancer cells to acquire resistance to anticancer drugs, termed multidrug resistance (MDR), remains a major problem to cancer therapy. The energy-dependent efflux of drugs mediated by ATP-binding cassette (ABC) transporters is one of the most investigated mechanisms. Overexpression of multidrug ABC transporters in cancer cells alters anticancer drugs efficacy by significantly reducing their accumulation inside the cell.

One of the lately discovered transporter to have a major role in MDR development is ABCG2. This transmembrane protein, was discovered simultaneously in three independent groups and then received different denominations: ABCP for its surexpression in placenta, BCRP for its isolation from resistant breast cancer cells, and MXR for its ability to confer resistance to mitoxantrone. ABCG2 has been demonstrated to confer resistance to a wide variety of anticancer agents.

Overcoming multidrug resistance (MDR phenotype) against anticancer agents is of critical importance for future clinical treatments. This strategy can be achieved through effective inhibitors of the multidrug ABC transporters involved in the MDR phenotype.

Herein, we describe the design, the synthesis and the biological studies (*in vitro* and *in vivo*) toward a new family of compounds that are highly potent, ABCG2 selective and non toxic inhibitors derived from acridones and chromones.

IL-206

COMPUTATIONAL MODELS OF CELL MOVEMENTS HOLD PROMISE FOR ASSESSING POTENTIAL PHARMACOLOGICAL INTERVENTIONS FOR CANCER METASTASES

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Cell and tissue movements play a crucial role in human embryogenesis, health maintenance, disease progression and therapy. Human conditions that are a direct result of irregular tissue motions include spina bifida, cleft lip and palate, cardiac septum defects, a host of adult malformation defects and cancer metastases. Many attempts have been made to use drugs and other therapies to prevent these serious conditions, but success has been limited. One of the reasons is that the mechanical interactions involved when cells and tissues move are quite complex. Cells may push or pull on each other and they may dissolve ECM and push neighboring cells apart in order to move. For such motions to occur, a variety of three-dimensional interactions must occur, multiple cell types must be involved, and the resultant forces must be just right. To properly understand these various interactions, and to determine the precise effects that potential drugs

would need to produce in order to control them, requires coupling of experiments and computational models. Previous computational and experimental studies by our research group revealed the conditions necessary for normal neural tube closure and showed that changes in gene expression, mechanical properties or drug interventions that affect force production by as little as 20% can produce neural tube defects such as spina bifida (HFSP Journal, 2010, doi: 10.2976/1.3338713). Other studies provided us with the tools to investigate cell·level rearrangements (ASME JBE, 2007, doi: 10.1115/1.2768375) and some of our most recent work (PNAS, doi:10.1073/pnas.1006591107) showed that slight force differences at the cellular level can profoundly affect medical outcomes. Here we combine these advances in order to investigate cancer metastases – specifically the conditions necessary for aberrant cells to leave a primary tumour. Through the use of two· and three·dimensional cell·level models, we show that cell surface properties are indeed a crucial factor in whether cells remain adhered to the primary tumour or wander from it and possibly seed secondary cancer sites. We can describe the precise mechanical conditions that are sufficient for cells to remain bound to the primary tumour and the conditions that are necessary for it to leave. The findings are consistent with a range of experiments. These studies also make it possible to identify specific changes that one would expect to see in cell contact angles and other geometric properties in histological sections if a drug or other intervention were bringing about the right kind(s) of changes in cells to fight metastases, even if the changes are not of sufficient magnitude to alter the medical outcome of the experiment. The assays to which the model leads make it possible to assess what fraction of the required change a particular therapy produces, thereby providing guidance for changing the intensity of that therapy or combining it with other strategies. This study suggests that computational models may serve an important role in assessing proposed pharmacological therapies.

IL-125

Track: *Innovative Drug Discovery and Nanotechnology*

COMBINATION OF QUANTUM FACTORS IN INTEGRAL MONO-PHARMACISTS AND THEIR ACTIONS IN CELLULAR REGENERATION AND TOTAL CURE

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Considering the classes of mono-pharmacists of integral medicine for the correction and restoration of sections of the energy bundle of the vital field [1], and its correspondents specialized mono-pharmacists for the entire cure of every organic system [1], there develop combinations of these mono-pharmacists that increase the curative total action, completing the quantum intelligence codes from the cellular level up to the organic level that are needed to recover the electronic memory [2], of each and every one of the organic systems of the human body.

Keywords: Cellular Regeneration, Integral Mono-Pharmacist, Organic electronic memory Quantum intelligence codes, Vital field.

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IL-185

THE FRONTIERS IN ANTIVIRAL THERAPIES: FROM BACK TO NATURE TO HIGH BIOTECH APPROACH

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Viral infections, like HIV-infection and influenza, are still estimated to cause the highest burden worldwide among the infection diseases. There is a continuing need for development of new antiviral drugs, drug combination and strategies, through methodical and scientific exploration of the enormous pool of synthetic, biological and natural products. As the need for new antiviral agents to effectively treat viral infections in low doses with no or mild side effects is constantly arising, biomedical research has evolved to facilitate efficient routes to effective agents for the treatment of AIDS, influenza, herpes, viral hepatitis, CMV and Rotavirus. Consequently, the research efforts in new drug development and

effiicient antiviral therapies are focused on one hand to find new, effective compounds either from the natural sources or by modifications for natural nucleosides, accordingly, with the objective to increase the therapeutic index of established antiviral agents. On the other hand, new approaches such as aptamer development and gene therapy in combination with stem cell research may offer highly efficient antiviral therapy, especially if taken as a personalised medicine approach. The potentials that can open new strategies in combating severe viral infections threatening to human in this era of emerging and re-emerging diseases will be discussed.

IL-5

CHALLENGES IN DRUG DISCOVERY – FROM NATURAL PRODUCTS TO DESIGNER MOLECULES

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We are well endowed today to create in the laboratory diverse arrays of new molecules with tailor-made structures and properties. In developing peptide based drugs, a common approach used to restrict the conformational degrees of freedom in small peptides involves designing structurally rigid non-peptide scaffolds which, when inserted in the appropriate sites in peptides, produce the specific secondary structures required for binding to their receptors leading to the development of potent agonists/antagonists. The number of reports on the development of constrained non-peptide scaffolds used in peptidomimetic studies is increasing rapidly. Newer concepts are emerging where the fundamental building blocks used by nature, like amino acids, sugars and nucleosides, are amalgamated to produce nature-like, and yet unnatural, de novo structural entities with multifunctional groups anchored on a single ensemble.

It is now well known that the secondary structural motifs so common in proteins are not restricted to the α-peptide backbone alone, but can be seen in many designer oligomers. Among the most studied families of non-natural oligomers that show interesting secondary structures are the β-, γ- and δ-peptides, which bear particular significance because of their similarity to α-peptides. We have developed many conformationally constrained scaffolds of sugar amino acids, furan and pyrrole amino acids, which belong to the family of γ- and δ-amino acids. They have been extensively used by us as peptide building blocks to synthesize many *de novo* peptides that have displayed interesting secondary structures and also useful properties like antimicrobial properties and binding with G-quadruplex. The presentation will give a brief overview of some of our latest results in these areas of research.

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IL-11

Track: *Hot Topics in Natural Products*

APPROACHES FOR THE TOTAL SYNTHESIS OF MARINE NATURAL PRODUCTS AS LEADS IN PHARMACEUTICALS

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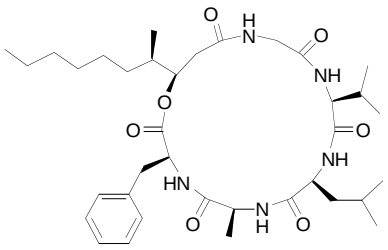
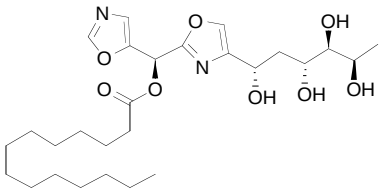
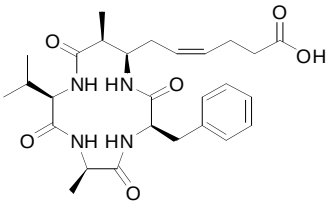
After an extensive search for new chemicals from terrestrial sources, especially for human health care, the researchers have turned their attention to marine sources. Very complex and interesting scaffolds are obtained in this unexplored territory of the universe. These diverse compounds even though are rich in biological profile could not be further explored due to scarce availability. Only total synthesis provided an alternative and the present lecture will focus on the efforts put up by our group in the total synthesis of some medicinally useful marine natural products (Bengazole, Arenamide, Azumamide E, Spongidepsin etc). The lecture also will highlight the importance of automation in analogue synthesis along with the biological profile of these compounds.



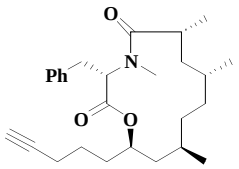
(*28R, 29R*) Arenamide A



Bengazole A



Azumamide E



(-)-Spongidepsin

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IL-129

Track: *Women's Health Drug Discovery and Therapy*

CONTROLLED MICROBICIDE DELIVERY USING INTRA-VAGINAL POLYMERIC MATRICES

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Intra-vaginal ring (IVR) devices are attracting increasing attention for local delivery of microbicides in the treatment and prevention of sexually transmitted infections (STIs) including AIDS. Antibiotic or antifungal agents were incorporated in matrices of polycaprolactone (PCL) at loadings of up to 15% (w/w) for Ciprofloxacin (CIP) and 3% (w/w) for Miconazole nitrate (MIC). Highly efficient drug release of 80 and 100% occurred in simulated vaginal fluid (SVF) in 30 and 14 days for CIP and MIC respectively. The release kinetics of MIC-loaded matrices can be described effectively by the Higuchi model. High levels of antimicrobial activity were retained by both CIP and MIC following release in SVF. Moreover, the predicted *in vivo* release concentrations are much higher than the minimum inhibitory concentrations against relevant STIs. PCL matrices having a 10% theoretical loading of the synthetic nucleoside analogue, acyclovir, displayed a cumulative release of 33% in 28 days in SVF. This behavior results in (*in vitro*) concentrations of antiviral from day 1 in excess of the IC_{50} value of 0.57μg/mL required for inactivating herpes simplex virus-2. The above findings recommend further investigations of PCL matrices in the form of IVRs for delivery of microbicides active against a broad range of STIs.

IL-107

Track: *Academic CRO/Industrial collaborations in drug discovery*

A NEW PARADIGM FOR PERSONALIZED MEDICINE AND COMPANION DIAGNOSTICS: THE CONTRACT DIAGNOSTICS ORGANIZATION

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In the era of personalized medicine, pharmaceutical companies are actively seeking partners to develop companion diagnostics. Common choices of partners are large diagnostics companies and traditional contract diagnostics organizations (CROs). However, diagnostics companies have their own product lines and may therefore choose to direct diagnostics development toward an existing technology platform, and the expertise of CROs lies in pharmaceuticals, not diagnostics. In addition, few traditional partners can offer accredited clinical laboratories to ensure that clinical trials for diagnostics are conducted in laboratories that meet regulatory standards. Lastly, there are numerous overall logistical complexities that arise when managing and coordinating multiple partners in the process.

The Contract Diagnostics Organization (CDO) is a new concept designed to assist pharmaceutical companies address challenges in diagnostics development. This business model allows pharmaceutical companies a complete partner to initiate the parallel development of companion diagnostic tests in synergy with drug development. The CDO combines all of the necessary services, including diagnostics research, a CLIA/CAP laboratory, manufacturing, and consulting, in an integrated, technology-independent manner. Thus, the CDO focuses on its pharmaceutical partner's business objectives and ensures the speediest path to market a valuable drug for patients.

IL-186

Track: *Regenerative Medicine*

RATIONAL DESIGN OF POLY (L-LACTIC ACID) SCAFFOLDS

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A Liquid-Liquid phase separation technique was used to make a bio-degradable poly (l-lactic acid) (PLLA) scaffold.1,2 The objective was to vary the freezing rates of the solution (PLLA in 1-4 dioxane) by using vials made of different materials (borosilicate glass, aluminium and copper), experimentally measure the temperature profiles experienced by the solution and to qualitatively determine the relationship between the imposed thermal history and the structure of the resulting scaffolds using Scanning Electron Microscopy (SEM) imaging.

The PLLA-dioxane solution was formed by dissolving 7% (wt) of PLLA in 1,4-Dioxane at 323K. The PLLA-dioxane solution was then frozen in three different vials with a constant volume of 5 ml. All the vials were 45 mm in height and 13.5 mm in diameter. The vials were inserted into a specifically designed polystyrene foam block and placed (floated) in a liquid nitrogen bath. The distance between the vials and the liquid nitrogen bath was carefully controlled and set at 1 inch. Thus, ensuring a controlled unidirectional freezing process. The thermal history experienced by the PLLA-dioxane solution in the three different vials were recorded using type-T hypodermic needle thermocouples (Omega Technologies, Stamford, CT, USA). Thermocouple voltages were read by a precision temperature data logger (Veriteq Instruments Inc, Richmond, BC, Canada) and transferred to a personal computer for further reduction and data analysis. The frozen solution was then placed in a freeze dryer (Model and Make) for 48 hours allowing the sublimation of dioxane. The resulting PLLA scaffold was analyzed using SEM to determine the relationship between the imposed thermal history and the resulting scaffold microstructure. The temperature-time graphs demonstrate that the imposed thermal history is quite consistent between various experiments in the glass and aluminium vials, but less so in the copper vials. This discrepancy can possibly be attributed to the high thermal conductivity of copper enhancing/aggravating the effect of minor variations in the experiments. The freezing point of the PLLA-dioxane solution was consistent at ~11.5°C. The SEM images indicate that PLLA scaffolds obtained using glass vials exhibited uniform porosity from the outer layer to the inner core. PLLA scaffolds obtained using aluminium and copper exhibited an increase in pore size from the outer layer to the inner core. However, PLLA scaffolds obtained using aluminium vials exhibited smaller pores at the outer layers while those obtained from copper vials exhibited extremely minute pores at the outer layers. The minute pores

exhibited in the copper vial PLLA scaffolds is probably a result of the high thermal conductivity of copper resulting in a rapid freezing process and the formation of smaller solvent crystals.

IL-112

Track: *Drug Delivery & Targeting*

ENHANCING THE EFFICACY OF DRUG-LOADED NANOCARRIER (DLN) AGAINST BRAIN TUMORS BY TARGETED RADIATION THERAPY

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Purpose/Objectives: The grim prognosis of many brain cancers is attributed, in part, to the inability to achieve therapeutic levels of anti-cancer drugs due to the impermeability of the tumor-associated blo od brain barrier (T-BBB). Novel drug delivery systems using nanocarrier polymers loaded with chemotherapy may increase serum half-life but are still limited by the T-BBB. Based on preliminary evidence that radiation therapy (RT) increases the permeability of the T-BBB, we developed a novel bioluminescent orthotopic mouse model of glioblastoma multiforme (GBM) to facilitate investigations of RT modulation of the T-BBB, and using this, we tested the efficacy of combined RT and paclitaxel drug-loaded nanocarrier (DLN) for treating GBM.

Material/Methods: U251 human-derived GBM cells expressing luciferase were established so that bioluminescent imaging (BLI) could assess tumor growth and response to treatment non-invasively and serially. These cells were injected into nude mice, either stereotactically into the brain or into the flank. The resultant mice with tumors were stratified into groups based on BLI signals to assess the efficacy of DLN and RT. Overall survival was calculated based on death or sustained loss of >20% of pre-treatment weight. BBB integrity was assessed via staining for extravasation out of the systemic circulation of IgG or fluorescent Evans Blue (EB) dye.

Results: We confirmed that RT disrupts the integrity of the BBB. Brains treated with 20 Gy RT showed substantial extravasation of IgG and EB ($p<0.05$) while unirradiated brains showed no extravasation. Intracranial tumors treated to 3 Gy x 4 showed increased T-BBB disruption compared to untreated tumors, with extravasation peaking at 20 days post-RT and gradually decreasing by days 35 or 55 ($p<0.05$). Flank tumors treated with DLN had significant delays in tumor progression ($p<0.05$) compared to untreated controls, but this effect was not observed in intracranial tumors that were identically treated --suggesting that the T-BBB limits the efficacy of DLN. In contrast, for mice with intracranial tumors, combined DLN + RT (3 Gy x 4) resulted in significantly delayed tumor growth ($p<0.05$) and significantly improved mean and median survival compared to mice receiving RT alone or controls (mean: 34 vs. 23 vs. 21 days; median: 34 vs. 21 vs. 21 days, $p<0.05$), suggesting a synergistic effect with RT + DLN. Dose-dependent efficacy of DLN was also noted.

Conclusion: The T-BBB is a potential impediment to the efficacy chemotherapy for treating intracranial tumors. Targeted RT however usefully disrupts the T-BBB and together with nanopolymerized paclitaxel DLN results in the greatest intracranial tumor response and extends survival in a preclinical model of GBM.

IL-188

Track: *Pharmaceutical Biotechnology*

RECOMBINANT TECHNOLOGY IN DRUG DISCOVERY AND THERAPY

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Recombinant technology is vital to drug discovery and therapy as specifically designed molecules can be created for personalized medicine based on molecules of plant and animal origin. Improved potency, long half-lives, enhanced bioavailability, and high yields are some of the many desired features of recombinant pharmaceuticals that have led to significant benefits. The increasing number of recombinant therapeutics in development that are brought to market is

reflective of recombinant technology as a powerful controlled biotechnology process that overcomes even the most challenging experimental, theoretical and regulatory aspects of drug discovery and therapy when meticulously implemented. Examples of such recombinant molecules include hypo/non-allergens against allergy, antibodies with antitumor efficacy, live vaccines that combat pathogenicity, nanoparticles in gene therapy, and probiotics for accurate targeting of therapeutics. As a research study, hypo/non-allergenic recombinant egg ovalbumin and its mutants were designed and developed, investigated for their protective efficiency against anaphylaxis in a mouse model through oral specific immunotherapy, and studied for their mechanisms of action. A double mutant and a triple mutant were effective in protecting mice against native ovalbumin/egg allergy/anaphylaxis, and hold significant potential in therapeutics and food safety. This paper presents the advances of recombinant technology in drug discovery and therapy.

IL-211

EFFECTS OF REACTIVE OXYGEN SPECIES AND RADIATION ON BIOMOLECULES

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The impact of reactive oxygen species (ROS, highly reactive radicals such as ·OH and O_2·-) and more stable species such as H_2O_2, e.g. produced by X-irradiation, and of UV radiation on many low-molecular and macromolecular compounds and fluids of biological interest has been investigated by various physicochemical techniques. In particular, spectroscopic studies of many proteins, polysaccharides and eye components, revealed, effectively and very fast, numerous changes of the local and global structure of the constituents under analysis, together with alterations of their functional ability.

By means of certain measures and a variety of additives (e.g. antioxidants), manifold modifications of the impact of ROS and of ionizing and nonionizing radiation can be achieved. Caused by differences in the primary reactions, biopolymers are protected effectively by typical ·OH scavengers against ROS and X-irradiation, whereas compounds exhibiting significant absorption behavior in the UV range ("chemical filters"), and, in some cases, also the O_2·- scavenger superoxide dismutase (SOD), turn out to act as potent protectives against UV light. A few substances provide protection against both sorts of radiation and are even able to provide a chemical repair of already damaged particles.

Combining the above results regarding damaging events and protection and repair possibilities of biomolecules and the professional know-how of radiation and photo chemistry provides the scientific basis for understanding the occurrence of a variety of effects of ROS and radiation on biomolecules on the molecular level, together with possibilities to avoid or modify the detrimental effects. The application of (bio)chemically relevant types and concentrations of additives (e.g. (di)thiols, ascorbate, formate, NADH, SOD) allows the suppression of the noxious effects of radiation to a large extent. The results obtained are also of importance for understanding and avoiding pathological alterations of biomolecules and for developing new strategies for the protection and repair of biomolecules, e.g., in context with the bioprotection of eyes and skin and the aging of biomolecules or the application and development of nutritional supplements and drugs. Currently, the finding of precautions to prevent or alleviate the damages caused by the Fukushima fallout (in particular of the long-living radioactive isotope Cs-137, a gamma-ray emitter) would be of utmost importance.

Keywords: Reactive oxygen species, irradiation, screening techniques, damages of biomolecules, protection and repair strategies, antioxidants.

IL-96

Track: *Academic CRO/Industrial collaborations in drug discovery*

SOMETHING OLD, SOMETHING NEW –SOMETHING BORROWED AND SOMETHING BLUE!? INNOVATIVE / RECYCLED- MODEL FOR DRUG DEVELOPMENT PARTNERSHIPS

Assem S. el Baghdady

Non-executive Chairman, AlphaBeta Pharma Group

It is apparent to all stake holders that the current models of drug development are not working. Major pharmaceutical companies and investors are failing everyone. In such a dire economic environment, an innovative formula is desperately needed, now more than ever.

The high overheads and exaggerated profit margins are shackling the industry and will not let go; pharmaceutical companies need to achieve astronomical profits in order to compensate for failed adventures and in the same time pushing "unfinished articles" ahead in order to maintain a healthy pipeline to inflate the share price to keep share holders happy and others at bay!

Investors -in turn- are demanding unrealistic return on investment in order to continue supporting this sector of the industry or they will venture elsewhere; to energy, communication or oil industries.

...and the vicious circle continues. Everyone is a winner except the patient!

An innovative model for drug development has to develop to push away the old unworkable ones and replace it with a sane, faire and productive model.

AlphaBeta Pharma was formed in order to do just that; returning to basics in research, development and partnerships, *hence the name AlphaBeta!*

We deal directly with the academic institutions, scientists and government bodies cutting the middle man and reducing costs and overheads to the minimum without compromising on science. Our partnership model is not new but it is innovative, in fact it is almost 1400 years old! in the middle east it is called "Musharakah"

AlphaBeta Pharma has recycled that model and introduced it to the industry as a sane, faire and productive model for the future.

Will the stakeholders receive that model well? What the investors think about it?

..and what do you think about it??

IL-52

Track: *Cardiovascular Drug Discovery & Therapy*

MITOCHONDRIA-SPECIFIC NANO-EMULSIFIED THERAPY FOR CARDIOVASCULAR PROTECTION AGAINST DOXORUBICIN—INDUCED OXIDATIVE AND NON-OXIDATIVE TOXICITIES

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The anthracycline, doxorubicin (DOX, Adriamycin) is a potent anti-neoplastic agent whose clinical use is limited by cumulative dose-dependent cardiac toxicity, in which mitochondrial damage is primarily implicated. Our work depicts the development of mitochondria-targeted nanoemulsions (NEs) containing tocopherol oxygen scavengers, and highly loaded with mitochondria-stabilizing therapeutics, namely Cyclospoine A (CsA). Our targeted nano-formulation, via TriPhenylPhosphonium (STPP) ligand, is capable of reaching target affected mitochondria in sufficient therapeutic concentration, in order to revert or at least limit oxidative and non-oxidative DOX-induced mitochondrial damage, manifested in affected cardio-vascular muscle tissues. Hence, Model rat cardiac muscle, H9C2 cardiomyocytes, and vascular media tunica media, A10, cells, were challenged with increasing concentrations of H2O2 and DOX, inducing both oxidative and membrane chaotropic effects on mitochondria, eventually leading to apoptotic muscle death. The Co-incubation with CSA-containing NE that were made specific to target mitochondria, via surface modification with STPP ligands, effectively inhibited cardiac and vascular cell death at almost all tested chemo-toxic concentrations of H2O2 (up to 1.25 mM), and DOX (up to 80μM) Using gel electrophoresis, DNA apoptotic laddering effect in H9C2 cells, induced by 25μM of doxorubicin was significantly prevented after co-incubation with STPPtargeted CSA-loaded NE. Moreover, Fluorescent microscopy tracking indicated marked mitochondrial co-localization of green FITC-labeled STPP-targeted NE and mito-tracker red-stained mitochondria, only after 2 hours of incubation, compared to control NE. Mitochondriotropic CSA NE overnight treatment resulted in almost 80% recovery of healthy mitochondrial membrane potential, using the JC-1 assay, after 4 hr exposure to 50μM of DOX, with subsequent significant inhibition of elevated levels of intrinsic apoptotic pathway marker, Caspase-9 enzyme, compared to all other treatment controls. Our targeted nano-therapy is capable of intracellular delivery of both therapeutic antioxidants and CsA, specifically and effectively preserving cardiac muscle mitochondria, acting as an adjuvant therapy to combat doxorubicin chemotherapy-induced cardiac failure. Such mitochondriotropic nano-therapy has the potential to expand the eligible patient profile, effective dose range, and spectrum of malignancies, eligible for treatment with potent adriamycin-based chemotherapy regimens. Moreover, our targeted platform carries significant prospects for treating closely-related oxidative damage molecular

mechanisms involved in ischemia-reperfusion injury, congestive heart failure, cardiomyopathies and acute cardiogenic shock.

IL-184

NOVEL GENISTEIN-LOADED LIPIDIC NANOCARRIER ADJUVANTS ENHANCE ANTICANCER EFFICACY AND OVERCOME CANCER RESISTANCE TO CHEMOTHERAPY

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Genistein, a small, biologically active flavonoid, is found in high amounts in soy, and is best known for its ability to markedly inhibit cancer progression, and metastasis. It has recently emerged as a Pgp drug-efflux pump inhibitor, as well as a potent apoptosis-inducing agent that occurs through disruption of mitochondrial membrane integrity, thus primarily triggering the intrinsic pathway of apoptosis, in different types of malignant cells.

Despite evident anti-cancer activity of genistein, its use is limited by its lipophilic nature, extremely low aqueous solubility, extensive metabolism and poor bioavailability and pharmacokinetics.

Our work describes the development and evaluation of new lipid-based nanocarriers (NCs), namely liposomes (Lip), nano-emulsions (NE) and polymeric phospholipid micelles (Mic), as drug delivery vehicles for improved both oral and parenteral delivery of genistein, via enhancing drug loading, and stability in biological systems. Hence, the therapeutic potential of genistein, either alone or in combination with other chemotherapeutic drugs, e.g. doxorubicin (DOX), against various types of cancers can be established.

Physico-chemically-screened Lips, NEs and Mics loaded with genistein were tested *in vitro* against murine sensitive breast carcinoma (4T1) and resilient colon cancer (C26). Inhibition of drug-efflux in human ovarian (OVCAR) and DOX-resistant (NCI/ADR-RES) cancers cells was assayed fluoromertically using drug-combination ratios with liposomal DOX.

Genistein-loaded vesicles showed high drug solubilization capacity (NE>Mic>Lip) and favorable nano-scale properties, leading to improved delivery of genistein, and subsequent superior cytotoxicity in tested cancers of different origins. Microscopic evaluation indicated enhanced uptake of the Lip drug into target colon carcinoma cells over 4 hours. Moreover, induction of morphological apoptosis features was quite significant after treatment of 4T1 with genistein-loaded Mic, compared to free drug and empty vehicle controls.

Superior cytotoxic effects were demonstrated for genistein Mic and Lip, in both 4T1 and C26 cancer cells, compared to treatment controls. Most importantly, IC50 values for genistein/DOX Lip were at least 3-6 folds < DOX-Lip in NCI/ADR-RES, showing significant synergistic 1:1 drug ratio, and additive 1:2, 2:1 and 3:1 drug ratios, respectively.

Genistein-loaded NCs showed high solubilization capacity and favorable nano-scale properties, leading to improved delivery of genistein to both naïve and drug-resistant cancer cells. These new genistein NCs can lead to therapeutic strategies using this potent pro-apoptotic nutraceutical, either alone or to augment the anti-neoplastic effect of doxorubicin and other susceptible drugs, in resistant tumors over-expressing drug efflux pump.

IL-6

BRCA1-IRIS OVEREXPRESSION PROMOTES FORMATION OF TN/BL AGGRESSIVE BREAST CANCERS

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Breast carcinomas negative for estrogen (ER) or progesterone (PR) and not associated with Her2/*neu* amplification are called triple negative/basal-like (TN/BL) breast cancers. These tumors represent 15-20% of all breast cancers and are prevalent in young, African American and BRCA1-mutant carrying women.TN/BL tumors are more resistant to current therapies resulting in disease recurrence and poor prognosis. We examined whether, the proto-oncogene BRCA1-IRIS can be a treatment target for TN/BL (or BRCA1/p220-associated) tumors.

Methods: We silenced BRCA1/p220 in normal human mammary epithelial (HME) cells or overexpressed it in several sporadic or BRCA1/p220 mutant breast cancer cell lines and correlated that to the expression of BRCA1-IRIS. We compared the expression relationship between BRCA1/p220 and BRCA1-IRIS and the expression of several basal markers and survival proteins. Using a newly generated mouse monoclonal anti-human BRCA1-IRIS antibody, we immunohistochemically stained breast tumorsamples andcorrelated that to expression of BRCA1/p220, AKT1, AKT2, p-AKT, survivin, ERα and PR. Where available, patients' tumor grade, tumor stage, age at diagnosis, and node positivity were also compared. We used HME cells overexpressing BRCA1-IRIS to generate subcutaneous tumorsin SCID mice and compared expression of basal markers, epithelial markers, AKT and survivin, as well as tumor phenotype and aggressiveness to Ras^{V12}-induced xenograft tumors.

Results: BRCA1/p220 silencing in HME cells reduced expression of two RNA-destabilizing proteins, namely AUF1 and pCBP2. Both proteins bind and destabilize *BRCA1-IRIS* mRNA, which explains BRCA1-IRIS overexpression in sporadic/low BRCA1/p220 expressing and BRCA1/p220-mutant tumor cells. Indeed, BRCA1-IRIS overexpression triggeredexpression of several TN/BL markers, e.g., cytokeratin 5 and 17, p-cadherin, EGFR and cyclin E as well as expression and activation of the pro-survival proteins AKT and survivin. Furthermore, BRCA1-IRIS silencing in the TN/BL cell line, SUM149 or restoration of BRCA1/p220 expression in the mutant cell line, HCC1937 reduced TN/BL markers, AKT and survivin expression and induced cell death. Moreover, the majorityof human breast tumors analyzed, especially $Her2^+$ and TN/BL tumors, were BRCA1-IRIS-positive. These tumors expressed high levels of AKT1, AKT2, p-AKT, and survivin, but were ERα-, PR-, and BRCA1/p220-negative. BRCA1-IRIS overexpressing TN/BL tumors were high-grade tumors, and showed lymph-node positivity and/or distant metastasis compared to BRCA1-IRIS-negative tumors. BRCA1-IRIS-positivitywas associated withearly age at diagnosisinpatients with TN/BL tumors compared to patients with the same tumors but negative for BRCA1/IRIS (48.9±10.1 vs. 70.8±6.8, $p \leq 0.01$). Moreover, HME cells overexpressing BRCA1-IRIS developed subcutaneous tumors in SCID mice, that were invasive and showed high expression levels of AKT1, AKT2, and p-AKT compared to Ras^{V12}-induced tumors. The aggressive BRCA1-IRIS-induced xenograft tumors expressed mesenchymal markers, e.g., vimentin, and not epithelial markers, e.g., CK19 and p63 and were BRCA1/p220-negative. In contrast, the less aggressive Ras^{V12}-induced xenograft tumors expressed CK19, p63, and BRCA1/p220, and not vimentin.

Conclusion: BRCA1/p220 loss promotes formation of aggressive TN/BL tumors by upregulating BRCA1-IRIS expression through a post-transcriptional mechanism. We propose that BRCA1-IRIS inhibition may be used as a novel therapeutic option for the treatmentof aggressive breast tumors of the $Her2^+$ and/or TN/BL subtype.

IL-189

Track: *"Anti-Cancer Drug Discovery & Therapy"*

GEMININ OVEREXPRESSION INDUCES IMATINIB SENSITIVE MAMMARY TUMORS VIA NUCLEAR C-ABL

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Despite the progress in our understanding of breast cancer progression, and in the development of novel therapeutic strategies, breast cancer remains the second leading cause of cancer-related deaths in women. Ten percent of patients have metastatic disease at the time of diagnosis and 20-50% of patients with primary tumors will eventually develop metastatic disease. Only ~25% of metastatic breast cancer patients survive beyond five years, which makes the research about breast cancer metastasis at the molecular and cellular level vital. We are also in dire need of new innovative ideas in basic and translational research to increase the survival rate of these patients. Accurate separation of the newly replicated DNA to daughter cells is crucial to maintain faithful transmission of genetic material from one generation to the next, and to prevent aneuploidy (abnormal chromosome content). Aneuploidy is observed in virtually all cancers, where it is considered a poor prognostic indicator, and an early event during breast cancer progression. Yet, we do not have a comprehensive understanding of how aneuploidy initiates during carcinogenesis. Aneuploidy can be caused by defects in mitotic spindle checkpoint, mitotic spindle assembly or cytokinesis. Aneuploidy can circumvent the necessity for cancer cells to undergo sequential genetic alterations in specific genes in order to get a clonal expansion advantage. We have identified a novel function for geminin in G2/M/early G1 cell as a cytokinesis inducer, which requires phosphorylation of geminin on 3 tyrosine residues at S-to-G2 trasnition. At normal levels, geminin is important for proper cytokinesis to occur, while when overexpressed, geminin causes cytokinesis skipping (failure) leading to genomic instability, aneuploidy in normal human mammary epithelial (HME) cells. In fact, geminin overexpression

transforms HME cells, in vitro and promotes tumor formation, in vivo, which support the notion that geminin may represent a novel breast cancer oncogene. Moreover, c-Abl kinase binds and phosphorylates geminin in G2/M/early G1 cells on tyrosine 150 and activates its cytokinetic function. Geminin and c-Abl are co-overexpressed in breast cancer cell lines and in ~50% of breast patient samples analyzed. Interestingly, geminin overexpression is only observed in human breast cancers expressing nuclear c-Abl. Furthermore, in tumors generated in mice using geminin overexpressing HME cells, c-Abl was also exclusively nuclear. Cells overexpressing Y150A mutant geminin or wild type geminin but exposed to imatinib (aka gleevec, c-Abl inhibitor) show no aneuploidy and do not form tumors in mice. In light of these findings, we propose that geminin is an inducer of breast cancer metastases through its ability to induce aneuploidy when overexpressed, and that the connection between geminin and nuclear c-Abl is a viable candidate for therapeutic intervention in breast (and other) cancer patients with geminin/nuclear c-Abl overexpressing tumors. We also propose that geminin overexpression maintains c-Abl nuclear, and that geminin/nuclear c-Abl overexpression could be used as a diagnostic tool to identify patients at increased risk of developing breast cancer metastases.

IL-21

Track: *Regenerative Medicine*

A PROSPECTIVE CASE SERIES OF PATIENTS TREATED WITH ADULT AUTOLOGOUS, CULTURE EXPANDED MESENCHYMAL STEM CELLS FOR SYMPTOMATIC OSTEOARTHRITIC HIP AND KNEE JOINTS COMPARED TO AN UNTREATED COMPARISON GROUP

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BACKGROUND: Adult autologous mesenchymal stem cells (MSCs) show prom ising potential as multipotent therapeutic agents for a number of orthopedic applications. In the present study, the authors describe the results of a percutaneous procedure of harvest, culture, and intra-articular reimplantation of MSCs in patients with symptomatic knee and hip osteoarthritis (OA).

METHODS: The study group was comprised of 202 patients consecutively seen at an outpatient facility; 148 for OA of the knee, and 54 for OA of the hip. Additionally, there were 25 untreated patient candidates (retrospectively contacted) who served as an unmatched control group. Patients underwent a bone marrow aspiration with isolation and culture expansion of MSCs using a serum free, autologous platelet lysate. Autologous MSCs were injected intra-articular utilizing fluoroscopic guidance. Percentage improvement, functional and visual analog scale data was collected via survey at 3, 6, 12, and 24 months.

RESULTS: At 12 months post procedure, both knee and hip patients reported more improvement than the untreated comparison group. The mean number of MSCs injected over all procedures for the knee and hip OA groups equaled 37.12 ± 32.29 million and 35.63 ± 36.18 million, respectively. The mean reported pain relief at one year was +60.07% in the knee OA group (n=70 reporting), +64.09% in the hip OA group (n=11 reporting), -3.15% (n=19) in the untreated knee comparison group, and –10.83% (n=6) in the untreated hip comparison group. The untreated comparison group values were collected an average of 1 year after first contact ($p<0.001$ for untreated vs. treated knee/hip groups). Significant decreases were seen in 4 out of 5 VAS score metrics and in most functional metrics in the knee group at 12 months post procedure. There were no serious complications reported among any of the treated patients.

CONCLUSIONS: Percutaneous implantation of cultured expanded MSCs into either a knee or hip joint was associated with sustained mean improvement among patients for as much as 2 years post-procedure. In comparison, members of an untreated control group reported average worsening of symptoms during a simultaneous 1-year follow-up period. The largest effects were observed in knee patients. Adult stem cell therapy of the joints using culture-expanded MSCs may offer a minimally invasive alternative to some surgical approaches to symptomatic knee and hip osteoarthritis. Further investigations with randomized treatment and placebo arm are warranted to further evaluate the results described here.

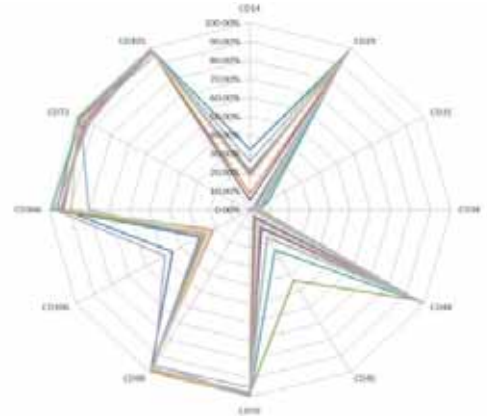

Fig. (1): The morphology grades used in culture expansion of MSCs. The goal in culture was to keepcells in the type A morphology with types B and C indicating culture stress.

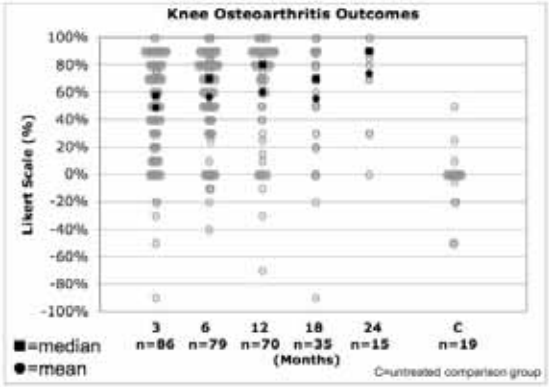

Fig. (2a). Likert scale pain relief data plotted by time point (dark grey line=median, light grey line=standard deviation).

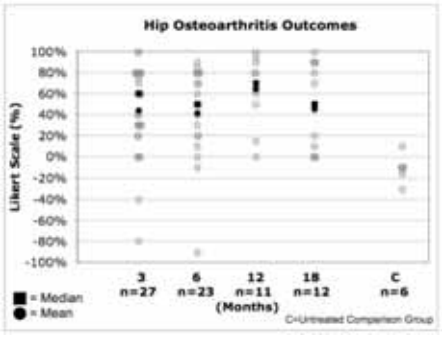

Fig. (2b). Likert scale pain relief data plotted by time point (dark grey line=median, light grey line=standard deviation).

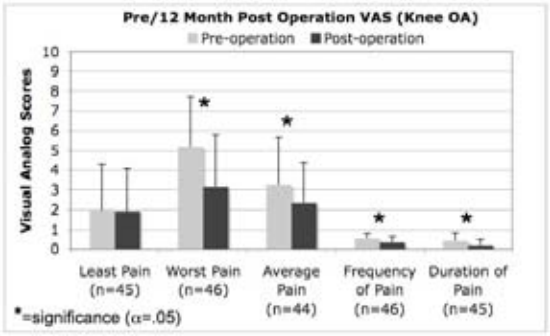

Fig. (3). Modified visual analog scale for knee OA before and after treatment.

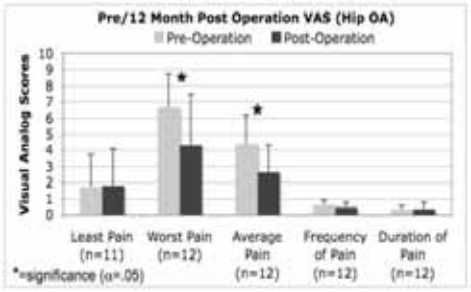

Fig. (4). Modified visual analog scale for hip OA before and after treatment.

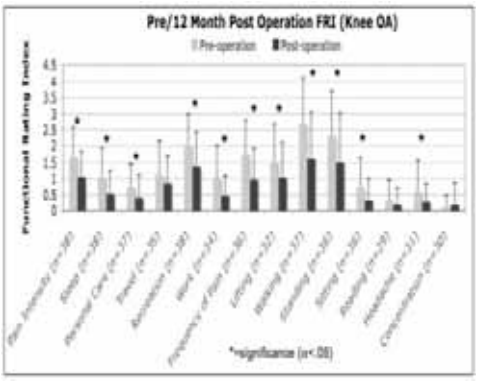

Fig. (5). Functional rating index subscales for knee before and after treatment.

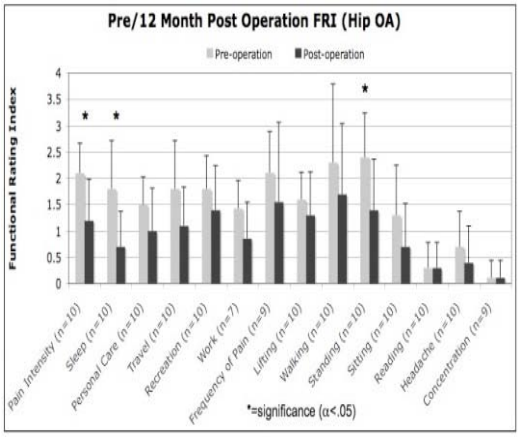

Fig. (6). Functional rating index subscales for hip before and after treatment.

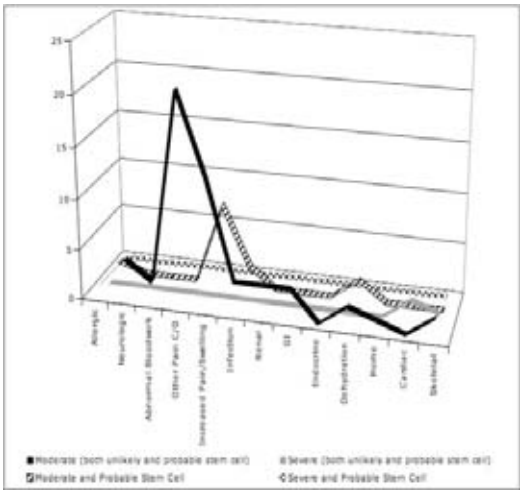

Fig. (7). Number of adjudicated complaints by body system: organized first by severity of all reports (either adjudicated as causally related or not to the procedure) and second by severity and adjudicated as probably related to the procedure. This includes all reports for dataset from 2006-2010. 32. Regenerative Medicinea. Stem Cells. Contact: SFaulkner@Regenexx.com

Keywords: Mesenchymal Stem Cells, Autologous, Osteoarthritis, Knee, Hip.

IL-164

Track: *Regenerative Medicine*

SAFETY AND COMPLICATIONS REPORTING UPDATE ON THE RE-IMPLANTATION OF CULTURE-EXPANDED MESENCHYMAL STEM CELLS USING AUTOLOGOUS PLATELET LYSATE TECHNIQUE

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Mesenchymal stem cells (MSCs) hold great promise as therapeutic agents in regenerative medicine. Numerous animal studies have documented the multipotency of MSCs, showing their capabilities for differentiating into orthopedic tissues such as muscle, bone, cartilage, and tendon. However, the safety of culture expanded MSC's for human use has only just begun to be reported [1].

Methods: Between 2006 and 2010, two groups of patients were treated for various orthopedic conditions with culture-expanded, autologous, bone marrow-derived MSCs (group 1: n=50; group 2: n=290-one patient in both groups). Cells were cultured in monolayer culture flasks using an autologous platelet lysate technique and re-injected into peripheral joints or into intervertebral discs with use of c-arm fluoroscopy. While both groups had prospective surveillance for complications, Group 1 additionally underwent 3.0T MRI tracking of the re-implant sites.

Results Section: The mean age of patients treated was 53 +/- 13.85 years; 214 were males and 125 females with mean follow-up time from any procedure being 435 days +/- 261 days. Number of contacts initiated based on time from first procedure was 482 at 3 months, 433 at 6 months, 316 contacts at 12 months, 110 contacts at 24 months, and 22 contacts at 36 months. For Group 1, 50 patients underwent 210 MRI surveillance procedures at 3 months, 6 months, 1 year and 2 years which failed to demonstrate any tumor formation at the re-implant sites. Formal disease surveillance for adverse events based on HHS criteria documented significantly less morbidity than is commonly reported for more invasive surgical procedures, all of which were either self-limited or were remedied with therapeutic measures. Two patients

were diagnosed with cancer out of 339 patients treated since study inception; however, this was almost certainly unrelated to the MSC therapy and the neoplasm rate in similar to that seen in the U.S. Caucasian population. Knee outcome data was collected on a subset of patients. Here, >75% improvement was reported in 41.4% while decreasing the improvement threshold to >50% improvement, 63.2% reported an improvement. At an average reporting time of 11.3 months from first procedure average reported relief in the knee sample equaled 53.1% (n=133 reporting).

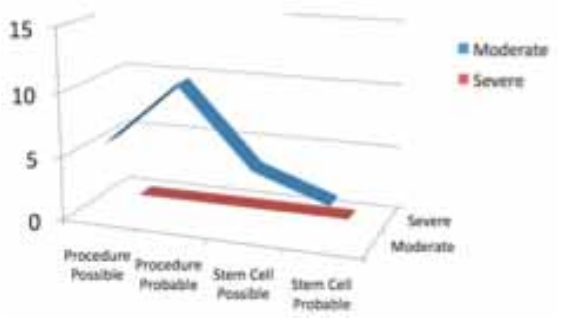

Figure 1. Number of adjudicated complications arranged by severity level and relatedness to either the re-implantation procedure or the stem cells (note that no severe level complications were adjudicated and that some patients complaints placed in both categories). This includes all reports for dataset from 2006-2010.

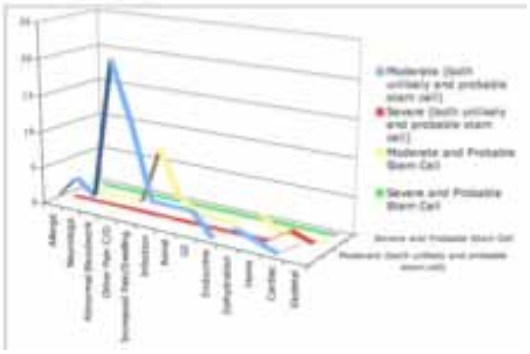

Figure 2. Number of adjudicated complaints by body system: organized first by severity of all reports (either adjudicated as causally related or not to the procedure) and second by severity and adjudicated as probably related to the procedure. This includes all reports for dataset from 2006-2010.

Discussion: Using both intensive high field MRI tracking and complications surveillance in 339 patients, no neoplastic complications were detected at any stem cell re-implantation site. These findings are consistent with our prior publication and other published reports that also show no evidence of malignant transformation *in vivo*, following implantation of MSCs for orthopedic use.

Significance: This present research supports the continuing trend that illustrates minimal complications reporting of culture expanded MSC's with autologous platelet lysate technique. This information provides groundwork for a critical step forward in educating patients in the risks involved. Furthermore, this study promotes discussion of this procedure as a verifiable consideration among patients seeking alternative orthopedic treatments.

Acknowledgements: Christopher Centeno and John Schultz performed the MSC re-implant procedures, Centeno also prepared the primary manuscript and reviewed and complied data. Michelle Cheever performed the cell culture and supervised the laboratory that was involved in cell culture. Stephen Faulkner and Jennifer Passerelli assisted in the analysis of the data and review of the manuscript. Brent Robinson and Ron Hanson assisted in manuscript review.

Reference:

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IL-126

Track: *Stereoselective Synthesis of Bioactive Compounds*

DEVELOPMENT OF DIMERIC FISCHER CARBENES: A BIDIRECTIONAL DÖTZ BENZANNULATION APPROACH TO DIMERIC PYRANONAPHTHOQUINONES

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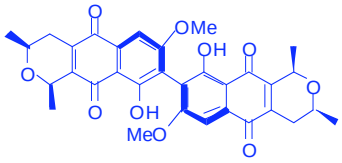
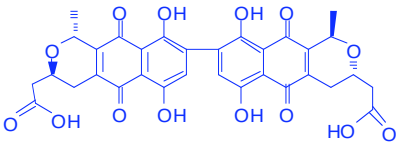
The antibiotic range and potency of quinones based on 3,4-dihydro-1*H*-naphtho[2,3-c]pyran is well established and their potential to act as bioreductive alkylating agents have spurred considerable interest in their synthesis [1]. Of the several known members of pyranonaphthoquinone natural products the dimeric molecules are of greater synthetic interest [1]. However the presence of axial chirality in some members and the dimeric skeleton posses greater challenges in their synthesis. Two approaches are

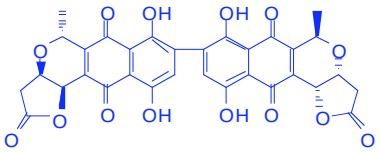
distinct from the literature: 1] a late stage homocoupling of monomer units to get the dimeric molecules and 2] a bidirectional built-up of the molecule. With a strong interest in bidirectional synthesis we designed dimeric Fischer carbenes as potential starting point. To demonstrate this we synthesized dimeric Fischer carbenes and completed the bidirectional synthesis of regioisomeric core of cardinalin 3 [2], and then extended the strategy to the total synthesis of (+)-demethoxy cardinalin 3 [3]. At present we are in final stages of the total synthesis of cardinalin 3, actinorhodin and gama-actinorhodin.



cardinalin 3



actinorhodin



γ-actinorhodin

References:

[1] M. A. Brimble, M. R. Nairn, H. Prabaharan, *Tetrahedron* **2000**, *56*, 1937.
[2] R. A. Fernandes, S. V. Mulay, *Synlett* **2010**, 2667.
[3] R. A. Fernandes, S. V. Mulay, *J. Org. Chem.* **2010**, *75*, 7029.

IL-99

Track: *Innovative Drug Discovery and Nanotechnology*

NANOMEDICINE AND INNER EAR

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One of the current initiatives in medicine is the exploitation of nanosized drug delivery vehicles, nanoparticles (NPs) to complement existing therapeutic strategies. NPs mediated drug delivery may offer increased efficacy and reduced drug-associated side-effects. The increased efficacy will occur in part as a consequence of the ability to target the drug, within the NP, to the site within the tissue where the therapeutic effect is required. Entrapment of the drug within the NP and controlled release at the required site may result in lower doses of drugs needed and hence reduced side effects. Targeting of the NPs require the identification of suitable ligands t o receptors which are selectively expressed at higher levels within the target tissue. Inner ear disease is a significant worldwide problem, with hearing loss affecting as many as 44 million people within the European Union alone. Strategies to prevent hearing loss are limited, and the cochlea is difficult to access by conventional systemic drug delivery due to the presence of the cochlea-blood barrier.

Cochlear implant is a treatment option for individuals with severe to profound hearing loss. These devices can be used effectively by both prelingually and postlingually deafened children and adults to perceive environmental sounds and speech. Nanotechnology offers new biocompatible nanomaterials and coatings that should increase the adhesion, durability and lifespan of implants.

Cell specific targeting to the inner ear cells by using a neurotrophin derived peptide ligand conjugated with nanoparticles would be a great deal for targeted drug delivery (Roy S. *et al*, 2010).

In a further study we report on the first *in vitro* tests of a new ferrogel consisting of supraparamagnetic iron oxide nanoparticle (SPIONS) and a Pluronic F127 copolymer. SPIONS were identified by light microscopy and localized with different imaging modes in energy filtered transmission electron microscopy (Thaler M. and Roy S. *et al.* 2011).

Furthermore we investigated the ability of polymersomes, lipid core nanocapsules and hyperbranched poly-l-lysine nanoparticles to cross the round window membrane. Nanoparticles were subsequently found to be distributed in the sensory cells, nerve fibres and other cells of the inner ear. In this investigation we were able to visualize nanoparticles across the round window membrane (Roy S. and R. Glueckert *et al.* 2011).

Activation of tyrosine kinase receptor B (TrkB) a neurotrophin receptor has been shown to increase neuronal cell survival and promote regeneration. Stimulation of TrkB receptor, by neurotrophic growth factors has been identified as a possible therapeutic target for the treatment of neurodegenerative disorders. We have conjugated hNgf-EE a short

peptide mimetic of NGFß to the surface of polymerosome nanoparticles and shown that they are capable of activating the TrkB receptor *in vitro* in a cell line (Roy S, *et al.* 2011).

These findings raises hope in terms of future multifunctional Nanoparticles –based drug delivery strategy in the human inner ear.

References:

Roy S., *et al,* 2010, Cell-specific targeting in the mouse inner ear using nanoparticles conjugated with a neurotrophin derived peptide ligand: Potential tool for drug delivery, International Journal of Pharmaceutics, DOI : 10.1016/j.ijpharm.2010.02.003.

Thaler M., Roy S., *et al,* 2011 Visualisation and analysis of supramagnetic iron oxide nanoparticles in the inner ear by light microscopy and energy filterd TEM. Nanomedicine : Nanotechnology, Biology and Medicine 2011 1-10 dii:1016 7jnano2010.11.005

Roy S., *et al.* 2011 Activation of TrkB receptors by NGFß mimetic peptide conjugated polymersome nanoparticles Nanomedicine : Nanotechnology, Biology and Medicine 2011 in Press

Roy S., and Glueckert R., *et al.* 2011 Strategies for drug delivery to the human inner ear by multifunctional nanoparticles Future medicine LTD in Press

This research was supported by European Community Grant number NMP4-CT-20060-26556)

IL-39

Track: *Translational Medicine*

PROTEASOME MODULATOR 9 IS LINKED TO MICROVASCULAR PATHOLOGY OF T2D

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The locus 12q24 is linked to type 2 diabetes (T2D) and to changes in retinal vascular caliber in Caucasians. Proteasome Modulator 9 gene (*PSMD9)* lies in the 12q24 locus and is implicated T2D onset and in degradation of intracellular proteins in antigenic peptides in the immune response to antigen presentation by MHC class I cells. Within *PSMD9*, we reported a linkage toT2D and to MODY3 in Italian families. We recently demonstrated a linkage of the *PSMD9* T2D risk single nucleotide polymorphisms (SNPs) with T2D-nephropathy, T2D-neuropathy, retinopathy, hypercholesterolemia, and macrovascular pathology.

We aimed at studying the presence of the linkage signal of the *PSMD9* SNPs *IVS3+nt460*, *IVS3+nt437*, *E197G* to microvascular pathology associated to T2D Italian siblings/families. We screened 200 T2D siblings/families for the *PSMD9* T2D risk SNPs and performed a parametric and non-parametric linkage study.

Our results show significant LOD score in linkage with microvascular pathology for the *PSMD9* SNPs studied using the non-parametric and parametric linkage analysis. The strongest signal is present under the recessive model. Our statistical power relies on the presence of T2D affected siblings, which represent an ideal dataset to identify linkage with a recessive disease model. Our simulation analysis confirms that the results are not due to random chance.

In summary, the *PSMD9 IVS3+nt460*, *IVS3+nt437*, *E197G SNPs* are linked via recessive model to microvascular pathology of T2D in Italians. A possible role of PSMD9 in microvascular pathology may be related to a causative pathogenetic role in inflammation as part of an autoimmune process.

IL-64

Track: *Cardiovascular Drug Discovery & Therapy*

PROTEASOME MODULATOR 9 AND DEPRESSION

Claudia Gragnoli

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Background: The chromosome 12q24 locus is linked to bipolar disorder, depression, and type 2 diabetes (T2D). *PSMD9* lies in the 12q24 locus and is linked MODY3, T2D, T2D-nephropathy, T2D-neuropathy, retinopathy, macrovascular pathology, hypertension, and hypercholesterolemia in Italian families. Interestingly, *PSMD9* gene common variants contribute to the therapeutic response to anti-depressant treatment.

Aims: Our goal was to determine whether *PSMD9* is linked to depression in Italian families.

Methods: We characterized the Italian families' members for presence and/or absence of depression using the diagnostic criteria of DSM-IV. The phenotype was described as unknown in all cases in which the diagnosis was unclear or data were not available. We tested in the 200 Italians families for evidence of linkage of the *PSMD9* single nucleotide polymorphisms (SNPs) *IVS3+nt460A>G*, *IVS3+nt437T>C* and *E197G A>G* with the depression phenotype. The non-parametric linkage analysis was performed by using the Merlin software. To rule out results due to random chance, 1000 replicates were executed.

Results: The PSMD9 gene SNPs studied and/or any gene variants in linkage disequilibrium with them are linked to depression in our Italian families.

Conclusions: This is the first report of *PSMD9* linkage to depression.

IL-173

Track: *Inflammation and Immunology*

TREATMENT WITH A PKC ALPHA/BETA INHIBITOR ATTENUATES HYPOXIA INDUCED INTERSTITIAL RENAL FIBROSIS AND INFLAMMATION VIA REDUCED ACTIVATION OF TGF-BETA SIGNALING

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Background – Ischemia reperfusion injury (IRI) leads to progressive renal fibrosis and loss of renal function. In this study we tested the efficacy of a PKC alpha/beta inhibitor (PKC-I) to attenuate post-ischemic renal fibrosis and inflammation.

Methods – IRI was induced in mice by transient unilateral clamping of the left renal pedicle for 45 min. Treatment with the PKC alpha/beta inhibitor (0.6mg/day) was initiated either prior to or 24h after ischemia and continued over 28 days. Renal morphology, glomerular filtration rate (GFR), renal blood flow (RBF), expression of alpha-SMA, collagen 4, and fibronectin were examined. Furthermore, expression of CTGF and PAI-1 down-stream targets of TGF-beta as well as inflammatory cell infiltration (macrophages, CD4+ T-cells) was investigated.

Results – In untreated mice GFR and RBF were significantly reduced 28 days after unilateral ischemia. In contrast, treatment with the PKC inhibitor improved renal function markedly. IRI caused severe renal fibrosis and up-regulation of pro-fibrotic proteins (a-SMA, fibronectin, collagen4). PKC-I pre-treatment partially blocked up-regulation of pro-fibrotic proteins. Furthermore, up-regulation of CTGF and PAI-1 expression was significantly ameliorated by PKC-I pre-treatment ($p<0.005$) and inflammatory cell infiltration was markedly attenuated.

Conclusion – PKC alpha/beta inhibition reduces experimental renal fibrosis via inhibition of TGF-beta signaling and attenuates renal inflammation.

IL-60

Track: *Anti-Cancer Drug Discovery & Therapy*

A NEW ANTI-CANCER DRUG AGAINST OVARIAN CANCER

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Ovarian cancer, difficult to diagnose at early stages (I,II) has poor prognosis at advanced stages (III,IV).

Two sesquiterpene lactones (SL's), EPD and EPA, were isolated from the leaves of a native Australian plant, *Calomeria amaranthoides*, using column chromatography and identified by ^{1}H-NMR and ^{13}C-NMR and mass spectrometry. To date steam distillation is used to isolate large quantities of pure compound.

Only EPD, eremophila-1(10)-11(13)-dien-12,8β-olide showed strong cytotoxic effects on ovarian cancer cells "*in vitro*" and "*in vivo*". Cytotoxicity of Cisplatin and Taxol were compared with EPD (range: 1 µg/ml -100 µg/ml) "*in vitro*" both on ovarian- and other cancer cell lines and normal cells (fibroblasts); cytotoxity "*in vivo*" was compared between Cisplatin (5mg/ml) and EPD (20mg/ml). EPD showed low cytotoxic effects towards normal cells, both Cisplatin and Taxol killed normal cells at low concentrations (1 to 5µg/ml) "*in vitro*". Cytotoxicity on ovarian cells of EPD and Cisplatin was comparable both "*in vitro*" and "*in vivo*". Nude mice, with ascites in the abdomen, were in poor condition after one month treatment with Cisplatin but not with EPD.

In conclusion: EPD is a potential anti-cancer drug for ovarian- and other cancers with low cytotoxic effects on normal cells.

IL-177

Track: *Diabetes and Obesity Drug Discovery & Therapy*

IMMUNOTHERAPY FOR TYPE 1 DIABETES: NECESSITY, CHALLENGES AND UNCONVENTIONAL OPPORTUNITIES

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Type 1 diabetes (T1D) is the leading autoimmune disease of childhood. T1D results from autoimmune destruction of pancreatic insulin-producing-ß-cells. Autoreactive T cells are activ ated by islet autoantigens in pancreatic lymph nodes (PLN) from where they infiltrate pancreatic islets of Langerhans to cause insulitis and ß-cell destruction. Although present within the normal repertoire of healthy individuals, such autoreactive T cells are held in check by suppressive cytokines and specialized subsets of regulatory cells. In disease-susceptible individuals and the widely used "non-obese diabetic (NOD) mouse model", these immunoregulatory mechanisms fail thereby permitting to diabetogenic T cells to infiltrate pancreatic islets, cause insulitis, and destroy ß-cells. What causes these mechanisms to fail and how the failures can be avoided/rectified are current questions of paramount scientific and clinical significance.

In this requested talk, I will discuss (1) why there is an urgent need of an immunotherapy for T1D despite success of insulin therapy in improving quality of life of patients and (2) why the limited efficacy of current therapies in phase I/II clinical trials has necessitated a quest for new approaches. In addition, we offer novel views on the potentials of targeting the Fas death pathway as an unconventional therapeutic approach for T1D that is unlikely to cause immune suppression.

The talk will discuss mechanisms that potently control ß-cell specific autoreactive T-cells when Fas ligand (FasL), an apoptosis-inducing member of tumor necrosis factor (TNF) family, is genetically or pharmacologically inactivated. Previously, the lack of both appropriate models and efficacious FasL-blocking mAbs has hampered such investigation. We are now using NOD mice that are haploinsufficient for FasL (NOD-gld/+ mouse) and a FasL-neutralizing mAb (MFL4 clone) to investigate the underlying mechanisms and therapeutic potentials of targeting FasL. NOD-gld/+ mice are completely protected from T1D and have normal immune homeostasis. In addition, MFL4 mAb protects NOD-wt mice from diabetes without altering immune homeostasis and importantly reversed diabetes in new-onset cases. As the

role of FasL in normal immune responses and ß-cell death are dispensable, understanding how it modulates the diabetogenic process may lead to new mechanistic insights with implications for developing safer therapeutic modalities.

On the basis of our published and new preliminary data, we hypothesize that IL-10-producing CD5+ regulatory B cells suppress diabetogenic autoreactive T-cells. In NOD-wt mice, FasL-mediated apoptosis eliminates IL-10-producing CD5+ regulatory B cells, thereby removing the brakes on autoreactive T-cells. In NOD mice, haploinsufficiency for FasL (gld/+) or mAb blockade of FasL prevents elimination of IL-10-producing CD5+ regulatory B cells leading to control of diabetogenic T cells and suppression of insulitis.

The discussion will be based on a book chapter" Type 1 Diabetes / Book 1", ISBN 978-953-307-362-0" to be published in July and on an in-Press paper in American Journal of Pathology to be published in July.

Keywords: Diabetes, immunotherapy and unconventional opportunities.

IL-55

Track: *Academic CRO/Industrial collaborations in drug discovery*

THE ROLE OF PATIENT ADVOCACY ORGANIZATIONS IN DRUG DISCOVERY AND DEVELOPMENT IN THE NEW ENVIRONMENT OF HEALTH CARE

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Voluntary Health Agencies (VHAs) and patient advocacy organizations are emerging as important participants in drug discovery. This new role has emerged as a consequence of several recent developments. Major pharmaceutical companies have found it advantageous to increasingly outsource the earliest stages of drug discovery. Academic centers are interested in expanding their capabilities in this area but need financial resources. Neither traditional federal research grants nor venture capital dollars can meet this need. Because they are driven by the interests of patients who are seeking solutions for their diseases, VHAs are in a unique position to harness philanthropic resources and are potentially important partners in bringing about this new approach to drug discovery. This talk will review some of the major needs that patients with arthritis have and provide a foundation for discussion of optimal investment strategies that VHAs might undertake in achieving better medicines.

IL-86

Track: *Drug Metabolism*

USING PK/PD TO DEFINE TARGET PROFILE EARLIER DURING DRUG DISCOVERY

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As an industry, we face a significant challenge in the translation of preclinical efficacy and safety model data towards proof of concept in the clinic, a main source of attrition. As such, there is a significant interest in applying mechanistic approaches that would allow more quantitative decisions towards new disease targets. One such approach is the successful implementation of Pharmacokinetic/Pharmacodynamic (PK/PD) in early drug discovery. PKPD can play a significant role in early preclinical drug discovery and can provide a framework for translational research which links, in a quantitative manner, the interactions between a drug (or combination of drugs), pharmacological targets, physiological pathways and, ultimately, integrated disease systems. The focus of this presentation will be to discuss approaches to better leverage PKPD (or quantitative pharmacology) across biological disciplines in order to more mechanistically describe disease. This may have impact in how we select targets, describe the mechanism of action, optimize our drug candidates and ultimately enhance our success in the clinic.

IL-67

Track: *Academic CRO/Industrial collaborations in drug discovery*

APOSCIENCE AG: CREATING AN INNOVATIVE REGENERATIVE MEDICINE COMPANY THROUGH A PUBLIC-PRIVATE PARTNERSHIP. AN AUSTRIAN EXPERIENCE

Rainer Henning

Chief Executive Officer, ApoScience AG

The regeneration of tissues and whole organs has recently become the focus of much attention, both scientifically and commercially. This broad interest was spurred by the advent of human embryonic stem cells, which are thought to have nearly unlimited potential in this regard. From a therapeutic realization standpoint even more fascinating was the discovery that a number of adult pluripotent stem cell populations, e.g. hematopoeitic and mesenchymal stem cells could be harvested from bone marrow and adipose tissue. These cells, when injected for instance into the heart following a myocardial infarction, were shown to reduce the amount of the organ damage and improve contractile function in animal models and subsequently in patients. However, it was soon discovered that this capability is not dependent on differentiation of these cells into muscle cells. The therapeutic effect is instead wholly dependent on the potent paracrine secretion of protective factors from these cells.

ApoScience was founded to commercially exploit the observation that the secretomes of cells undergoing apoptosis have very potent anti-inflammatory and pro-angiogenic activity and stimulate cell survival pathways, and may be superior to adult stem cells. We could show in rodent and porcine models that secretomes from apoptotic peripheral leukocytes (APOSEC®), which can be procured by a very simple process, improve cutaneous wound healing, reduce infarct size and promote functional recovery in acute and chronic myocardial infarction, and reduce lesion size in ischemic stroke. Additional multiple disease applications are currently investigated. The company plans to enter into clinical trials in an orphan disease with chronic skin lesions within 2012.

Financing start-up companies with advanced technology has become very challenging, especially in Europe. ApoScience benefited from a public private partnership model unique to Austria, where private investors and the federal government through the Christian Doppler Society work together to facilitate early stage translation of exiting science to commercial products. In the case of successful proof of concept, the company will then be able to attract more classical financing for product advancement.

IL-79

USING PUBLIC INVESTMENTS IN RESEARCH OPTIMALLY IN SUPPORT OF INDUSTRY

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Although industry working alone has demonstrated the strong ability to develop many new and marketable pharmaceutical products, extensive experience in many industrial economies over the last 150 years has shown that the most productive path for creating new entirely approaches to the diagnosis, treatment and cure of diseases is by research conducted in public and private research universities. In creating this optimally creative and productive environment three main factors have proven to be central to success. The first is the creative interaction between faculty members and students, particularly graduate students. Students in a rich, education and research learning environment are encouraged to question, and in particular to differ in their opinions with faculty mentors at both the undergraduate and graduate levels. It is clear that the most creative environments for discovery flourish in societies where questioning and evaluation by students are encouraged, supported and rewarded. The second key factor is the intellectual rigor and discipline created by the need for research teams to compete for research funding against standards of excellence created by the limited availability of funds and peer review of proposals. The key third factor is close communication between academic research teams and industry, facilitated by skilled manages of intellectual property. Defining appropriate parameters of intellectual property ownership and rights to create commercial partnerships in exploiting intellectual property through appropriate legislation are at the heart of establishing the relationships that create new and successful industry.

IL-204

Track: *Anti-Cancer Drug Discovery & Therapy*

PLATINUM RESISTANCE IN OVARIAN CANCER AND THE WAYS TO OVERCOME IT

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Although cisplatin and its analogues carboplatin and oxaliplatin are widely used in the clinic, their use has also been limited due to intrinsic and/or acquired resistance (thus limiting the spectrum of activity) and a number of side effects. With the idea that the difference in nature of binding with DNA may translate into an altered spectrum of activity, rule-breaker platinum compounds have been prepared in our laboratory and elsewhere with the aim of widening the spectrum of activity. Thus, we have synthesized a number of highly tumour active novel platinum compounds with or more metal centres. These include compounds coded as YH9, YH10, YH11. YH12, CH1, DH6Cl, TH1 and QH1. Some of the compounds are found to display much greater activity than cisplatin especially in the cisplatin-resistant tumour models.

Knowing that combination chemotherapy using drugs with different mechanisms can offer a means of overcoming drug resistance, we have used binary combinations of cisplatin (CS), oxaliplatin (Ox) and designed platinums YH12, CH1 and TH1 and a number of phytochemicals including: paclitaxel (Tax), colchicine (Col), curcumin (Cur), epigallocatechin-3-gallate (Egcg), chlorophyllin (Chl), andrographolide (Andro), thymoquinone (Thy), resveratrol (Res), betulinic acid (Bet) and ursolic acid (UA) were applied to three human ovarian cancer cell lines A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$ as a function of concentration and the sequence of addition. Both the dose effect curves and combination indices show that the binary combinations of platinums with phytochemicals show concentration- and sequence-dependent synergism in the cell lines. Generally the degree of synergism is found to be greater in sequenced additions such as 0/2 h, 2/0 h, 0/4 h and 4/0 h than the bolus. The change in synergism with the change in sequence of addition clearly indicates that the action of one drug modulates that of the other (towards the induction of apoptosis). We have also used sequenced combinations of platinum drugs and bortezomib (a proteasome inhibitor that prevents cisplatin-induced proteasomal degration of copper transporter CTR1) to enhance cellular platinum accumulation and the level of platinum-DNA binding especially in the resistant human ovarian tumour models. In addition, we have administered platinum drugs in two aliquots with a time gap to optimise cell kill. We have carried out proteomic studies to identify key proteins associated with platinum resistance. The studies have identified five proteins namely TCTP, RSSA Human, MARE1_HUMAN, ANXA5, ATPB, that are believed to be associated with platinum resistance in ovarian cancer cell line $A2780^{cisR}$. Synergistic combination of CS and Tax is found to restore expressions of TCTP and ATPB, that of CS and Col is found to restore expressions of ANXA5 and ATPB, and that of CS and Egcg is found to restore expressions of TCTP and ANXA5.

IL-178

ANTIBODY PHAGE DISPLAY FOR DIAGNOSTICS AND THERAPY

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Antibody phage display is an essential tool to generate monoclonal (mainly human) antibodies for proteome research, diagnostics and therapy. For this purpose three human, naive antibody gene libraries (HAL4/7/8) were constructed and a high-throughput compatible antibody selection pipeline including a transient ma mmalian expression system was set up. The quality of the libraries and the selection pipeline was validated with more than 150 antigens, including human, other mammalian, fungal or bacterial proteins, viruses or haptens. In this presentation, our antibody generation pipeline will be shown. Furthermore, examples for the application of recombinant antibodies from this pipeline for diagnostics of toxins (Botulinum toxins), bacteria (*Salmonella typhimurium*) and fungi (*Aspergillus fumigatus*) and therapy (breast cancer and Anthrax) are given.

IL-65

Track: *Drug Discovery in Preclinical Research*

PHAGE DISPLAY FOR THE SELECTION OF IMMUNOGENIC PROTEINS FOR DIAGNOSTICS AND VACCINE DEVELOPMENT

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Phage display of oligopeptides of bacterial pathogens allows the identification of immunogenic proteins which may cannot be discovered by classical methods like 2D PAGE of cultured bacterial pathogens, immunoblot using sera from of infected patients or animals followed by mass spectrometry or microsequencing. Here, we demonstrate a powerful selection system for novel immunogenic antigens based on the display of protein fragments from genomic libraries of pathogens on M13 filamentous phage. Furthermore, this technology also includes a unique selection of open reading frames (ORF) during phage production to enhance the quality of the library, the selection and screening process. Examples for the application of this technology are given for the identification of immunogenic antigens of *Mycoplasma hyopneumniae* and *M. mycoides*. The selection of immunogenic proteins is linked with our pipeline for the generation of recombinant human antibodies using phage display. Here, we will present the identification of new immunogenic proteins of *Salmonella typhimurium* and the generation of human antibodies against these antigens using phage display for the development of vaccines and diagnostics. In addition, the use of oligepeptide phage display for the identification of the epitopes of GP41 neutralising antibodies of HIV-1 elite controllers.

IL-4

Track: *CNS Drug Discovery & Therapy*

CISPLATIN, A HSP90 INHIBITOR

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The 90-kDa heat shock protein (HSP90) acts as a specific molecular chaperone in the folding and regulates a wide range of associated proteins such as steroid hormone receptors. It is known that HSP90 possesses two different chaperone sites, both in the N- and C-domains, and that the chaperone activity of HSP90 is blocked by binding of geldanamycin (GA) to the N-domain, the same as the ATP-binding site. Here we show that Cisplatin [cis-diamminedichloroplatinum (II), CDDP], an antineoplastic agent, associates with HSP90 and reduces its chaperone activity. In order to analyse the binding proteins, bovine brain cytosols were applied to a CDDP-affinity column and binding proteins were eluted by CDDP. In the elutants, only 90-kDa protein bands were detected on SDS/PAGE, and the protein was cross-reacted with the anti-HSP90 antibody on immunoblotting. No protein bands were detected in the elutants from the control column on SDS/PAGE. These results indicated that CDDP has a high affinity for HSP90. On CD spectrum analysis, the binding of CDDP to HSP90 resulted in a conformational change in the protein. Although HSP90 inhibited the aggregation of citrate synthase as a molecular chaperone *in vitro*, the activity was suppressed almost completely in the presence of CDDP. Mg/ATP has an influence on the chaperone activity to some extent. The CDDP binds both the amino terminal and carboxyl terminal domains of the human HSP90 and differently affects these two domains. Cisplatin blocks the aggregation prevention activity of HSP90C, but not HSP90N. In contrast, cisplatin induces a conformational change in HSP90N, but not HSP90C. These results indicate that cisplatin modulates the HSP90 activities through two different mechanisms using the two distinct binding sites of the HSP90 molecule.

Our results suggest that the chaperone activity of HSP90 may be inhibited by the binding of CDDP or GA by different mechanisms.

IL-100

Track: *Translational Medicine*

HUMAN SERUM TRANSTHYRETIN LEVELS CORRELATE INVERSELY WITH ALZHEIMER DISEASE

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Alzheimer's disease (AD) is the most common form of dementia and the fastest growing neurodegenerative disease in the elderly population. There is no definitive cure for this disease and current therapeutic medicines may help with some of the symptoms, such as cognitive impairment and behavior disorder, or delay the progress of disease. Therefore, the search for therapeutic targets and diagnostic AD biomarkers is an exigent issue. Amyloid β (Aβ) aggregation constitutes the epicenter of AD pathology, Aβ-binding proteins that regulate Aβ aggregation, such as transthyretin (TTR), have attracted much attention. TTR itself maintains normal cognitive function during aging. In addition, TTR binds to Aβ, prevents its aggregation, and consequently inhibits Aβ-induced cellular toxicity. Decreased TTR levels in cerebrospinal fluid (CSF) from AD patients suggest that TTR is a biomarker of AD. But, studies on TTR as a biomarker have focused on CSF; no definitive investigation has been performed in peripheral levels of TTR in AD. Since serum has many appealing features as biomarker including easy detection and routine accessibility, we examined the correlation between serum TTR levels and AD. We measured TTR levels in serum samples from non-demented elderly subjects (n=90) and clinically diagnosed AD patients (n=111) by the method of ELSIA. Differences in serum TTR levels between control and AD subjects were analyzed based on age and gender. TTR levels correlated significantly with AD and gender but not with age. We observed a significant decrease in serum TTR levels in AD ($p< 0.001$) compared with non-demented controls. Females in the control group had lower serum TTR levels compared with male in the control ($p=0.006$), while no difference in gender was noted in the AD group. According to 3-way ANOVA, TTR levels correlated significantly with the AD patient group ($p<0.001$), group-age interaction ($p=0.001$), gender-age interaction ($p=0.001$), and the group-gender-age three-way interaction ($p=0.006$). This study demonstrates a clear negative correlation between serum TTR levels and AD, suggesting that TTR is not only involved in AD pathological process but also suggested as a possible peripheral biomarker for AD diagnosis in serum level.

Keywords: Alzheimer's disease, amyloid-β aggregation, biomarker, serum, transthyretin.

IL-95

THE O6-ALKYLGUANINE RESPONSE: MECHANISMS AND IMPLICATIONS FOR CANCER THERAPY

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First line therapy in the treatment of gliomas and malignant melanomas are alkylating agents. These agents induce a dozen different DNA lesions, some of them have been identified to be carcinogenic, genotoxic and cytotoxic. A critical DNA adduct is O^6-methylguanine (O^6MeG). This damage causes mutations and is responsible for most of the carcinogenic effects of alkylating agents. At the same time O^6MeG is a highly powerful cytotoxic lesion giving rise to apoptosis, necrosis and autophagy induction. The damage is repaired by the suicide enzyme alkyltransferase (MGMT), which is a very important defense mechanism and marker of alkylating drug resistance, notably in malignant glioma. For malignant melanoma the situation is less clear; very likely other mechanisms of alkylation drug resistance come into play as well, such as mismatch repair and regulators of apoptosis. O^6MeG is a potent trigger of apoptosis. We have studied in detail how apoptosis is induced following O^6MeG and executed in glioma and melanoma cells. We have shown that O^6MeG triggered cell death is executed via the death receptor and the mitochondrial damage pathway, involving DNA double-strand breaks (DSB). The major pathway of repairing O^6MeG and O^6-chloroethylguanine (induced by the anticancer drugs ACNU and CCNU) triggered DSB is homologous recombination (HR). Therefore, players involved in DSB recognition and HR are potential targets for therapy, such as NBS-1, ATM, Rad51, XRCC2 and XRCC3. In glioma, the efficiency of O^6MeG to trigger the p53 dependent death receptor pathway is higher than the p53 independent endogenous mitochondrial pathway, which explains why p53 wt glioma cells are more sensitive to

temozolomide than p53 mutated cells. Interestingly, p53 wt glioma cells are more resistant than p53 mutant glioma cells to chloroethylating agents (CCNU, ACNU), which are also applied in glioma therapy. This indicates that p53 has a dual role: one is the up-regulation of the death receptor thus sensitizing to methylating agents, the other is the upregulation of DNA repair genes such as *ddb2* thus protecting against O^6chloroethylguanine-induced apoptosis (and necrosis). Melanoma cells display a high intrinsic level of resistance to temozolomide, which is due to silencing of caspase-8 and induction of DNA repair. Strategies aimed at abrogating intrinsic drug resistance will be discussed, including interferon and valproic acid co-treatment that reactivate the apoptotic pathway. Work was supported by DFG KA724 and Deutsche Krebshilfe.

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IL-80

Track: *Drug Metabolism*

DEUTERATED DRUG APPROACH TO MITIGATE FORMATION OF REACTIVE METABOLITES DURING DRUG DISCOVERY

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Idiosyncratic drug reactions (IDRs) are a major impediment in drug development. Although the precise mechanisms of IDRs remain unclear, circumstantial evidence suggests the involvement of reactive metabolites (RMs) in these reactions. As part of lead optimization efforts in drug discovery process, the potential for RM formation is routinely examined in order to assess and mitigate the risk of idiosyncratic drug toxicity. Deuterated drug approach can potentially lead to a variety of beneficial effects, including reduced levels of toxic metabolites and/or deflect metabolism away from pathways leading to metabolites with toxic properties and thus may lead to improved safety profile. Examples (from literature and own work) are presented to illustrate how this approach has been applied with emphasis on mechanism of the formation of reactive metabolites that may lead to toxic effects.

IL-183

Track: *Pharmaceutical Biotechnology*

CREATION OF UNIVERSAL VECTORS FOR PROPHYLACTIC AND/OR THERAPEUTIC RECOMBINANT VIRUS VACCINES

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Vaccination against infectious agents has proven to be the best way to prevent infectious diseases. We have created genetically modified recombinant M gene mutant of the Indiana serotype of vesicular stomatitis virus (VSVInd) and M gene mutant of the New Jersey serotype of VSV (VSVNJ) as universal vectors for the development of recombinant virus vaccines. The priming vaccine vector should be antigenically distinct from the boost vaccine vector in order to maximize the boost effects. rVSVInd with the mutations of G21E/M51R/L111F in the M protein (VSVIndGML) and rVSVNJ with the mutations of G22E/M48R+M51R in the M protein (rVSVNJGMM) was attenuated to a degree that mice injected with 50 million of these genetically modified infectious viruses directly into the brain showed no neurological signs or any other adverse effects. In contrast, 100 infectious wild-type VSVInd or wild-type VSVNJ kills mouse within 48 hours. Foreign genes inserted into these VSV vectors elicit strong B cell and T cell immune responses against the inserted gene products when we prime animals with VSVInd(GML) followed by boost immunization with rVSVNJ(GMM) carrying the same genes of interest. Animals can tolerate more than 5 x 109 PFU each of recombinant infectious VSVInd(GML) and recombinant infectious rVSVNJ(GMM) and showed high levels of gene expression and immune responses. Our results show clearly that rVSVInd(GML) priming and rVSVNJ(GMM) boosting is the best way to induce ultimate humoral and

cellular immune responses. We will describe the advantages of these dual serotype VSV vectors for future vaccine development against infectious diseases and cancers.

IL-45

Track: *Hot Topics in HIV Research*

INTRINSIC RESTRICTION ACTIVITY BY APOBEC ENZYMES AGAINST HIV AND RETROELEMENT

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A large portion of the mammalian genome is derived from ancient transposable elements. Retroelements, transported by an intracellular copy-and-paste process involving an RNA intermediate(retrotransposition), constitute a majority of these mobile genetic elements. Endogenous retroviruses are LTR-type retroelements accounting for around 8% of human or murine genomic DNA. Non-LTR members are present in extremely high copy numbers; with LINE-1 contributing to nearly 40% of human and murine genomes. These LINE-1 elements modify mammalian genomes not only through insertions, but also by indirect replication of nonautonomous retrotransposons such as SINEs. As expected, cellular machineries of vertebrate's innate immunity have evolved to support a balance between retroelement insertions that cause deleterious gene disruptions and those that confer beneficial genetic diversity. The ability of mammalian cytidine deaminases encoded by the *APOBEC3* (*A3*) *genes* to restrict a broad number of endogenous retroelements and exogenous retroviruses, including HIV-1, is now well established. The RNA editing family member apolipoprotein B (apo B)-editing catalytic subunit 1 (APOBEC1; A1) from a variety of mammalian species, a protein involved in lipid transport and which mediates C-to-U deamination of mRNA for apo B, has also been shown to modify a range of exogenous retroviruses, but it's activity against endogenous retroelements remains unclear. Here we show that A1 family proteins can also reduce the mobility and infectivity potential of LINE-1 and LTR retrotransposons (or endogenous retroviruses) such as IAP and MusD sequences. The anti-L1 activity of A1 was mainly mediated by a deamination-independent mechanism with inhibition at step prior to the integration, and was not affected by nuclear localization of the proteins. In contrast, A1 inhibits the replication of murine IAP, MusD as well as HIV-1 through a DNA deamination-dependent mechanism. Thus, the APOBEC family including A1s employ multiple mechanisms to regulate the mobility of autonomous retrotransposons in a wide range of mammalian species. The ability of *APOBEC1* -/- knock-out mice to support the retrotransposition of retrovirus and retroelement are currently under investigation and will be discussed.

IL-47

Track: *Innovative Drug Discovery and Nanotechnology*

POTENTIAL REMOVAL OF CIRCULATING TUMOR CELLS BY PHOTOTHERMOABLATION USING GOLD-NANOPARTICLE CONSTRUCTS

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Background: Metastasis is the final event of clonogenic tumor cells being released from the primary tumor, enter circulation, extravasate into perivascular tissue at a distant site and proliferate as a secondary tumor colony. The number of circulating tumor cells (CTCs) in patients with metastatic breast cancer before treatment has been shown to predict progression-free and overall survival [1]. Therefore, reducing or even eliminating CTCs in patients with advanced breast cancer or other malignancies such as prostate, colon and lung cancer may significantly lower the risk of clonogenic tumor cell engraftment at a site distant from the primary tumor site (metastasis), and thus may benefit progression-free survival or overall survival in patients with advanced stage malignancy or in patients undergoing tumor removal surgery.

Treatment Concept: The treatment concept of reducing/eliminating CTCs is based on identifying CTCs by monoclonal antibody conjugated gold nanoparticles. These tumor cell-monoclonal antibody-gold nanoparticle constructs are targeted, while passing through an extracorporeal circulation, by a near-infrared (NIR) laser device, and eventually eliminated through heat absorption and subsequent destruction.

Clinical Requirements: CTCs are targeted in an extracorporeal pump-assisted circulation. Photothermal ablation is accomplished by an NIR opposing dual laser light designed to operate in the 650 to 900 nm wavelength (so called NIR window). To guarantee homogenous laser light distribution cells pass continuously through a monolayer tubing device while exposed to NIR laser light. During one treatment session CTCs can be targeted multiple times whenever they pass the extracorporeal circulation.

CTCs expressing epithelial cell adhesion molecule (EpCAM) are identified by systemically administered anti-EpCAM conjugated gold nanoparticles such as gold-silica nanoshells, gold nanorods or gold nanocages. Other options include the use of anti-epidermal growth factor receptor (EGFR) antibody and anti-HER2/neu antibody conjugated gold nanoparticles.

The design of a clinical photothermoablation device using gold nanoparticles in a continuous flow configuration depends on certain criteria that determine sufficient heating and subsequent killing of CTCs, such as NIR light intensity, absorptive cross-section of the gold nanoparticle, concentration of nanoparticles on the cell surface, time of exposure to NIR laser light, difference in heat absorption of gold nanoparticles and surrounding plasma (heat sink), and negligible toxicity to cells not attached to gold nanoparticles.

Conclusions: Gold nanoparticle photothermoablation of CTCs in an extracorporeal circulation is an attractive treatment option for patients in advanced stage malignancy who are at risk for metastasis.

Keywords: Gold- nanoparticles, circulating tumor stem cells, cancer stem cells, near-infrared laser light.

Reference:

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IL-163

Track: *Inflammation and Immunology*

CpG-ODN INDUCES CD274 EXPRESSION ON HUMAN B CELLS AND CpG ODN -TREATED B CELLS DECREASED IL-5 PRODUCTION FROM ANTIGEN-STIMULATED HUMAN CD4+ CELLS

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Background: CD274 (PD-L1, B7-H1), a member of costimulatory/co-inhibitory ligands that is expressed on immune cells, has emerged as an important immune modulator that is capable of blocking T cell receptor signaling.

Objective: We investigated the effect of CpG-ODN on the human B cells *in vitro*. The expression of co-stimulatory molecule of B cells and its function were analyzed.

Method: The human B cells were cultured and stimulated with CpG-ODN and the expression levels of CD274 and other co-stimulatory molecule ligands were detected by real time PCR. We also investigated the effect on cytokine-production from antigen-stimulated human CD4+ cells when CpG-ODN-treated B cells and CD4+ cells were co-cultured.

Conclusion: CpG ODN increased PD-L1-expression. CpG ODN-treated B cells decreased IL-5 production from antigen-stimulated human CD4+ cells. These results suggested that the treatment of CpG-DNA suppressed antigen-specific IL-5 production via PD-1- PD-L1 ligation. This study reinforces the idea of CpG-DNA being a potential therapeutic modality through B cells and its signaling pathway being a target for drug interventions against allergic diseases.

Keywords: CpG, PD-L1, CD274, B cell, costimulatory molecule.

IL-201

Track: *Biologics*

A NEW ROLE OF ENDOTHELIN-1 AXIS IN THE INVASIVENESS OF TRIPLE-NEGATIVE BREAST CANCER PHENOTYPES

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Triple-negative breast cancer is characterized by the lack of expression of estrogen receptor-α (ER-α), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER-2). However, pathways responsible for downregulation of therapeutic receptors, as well as subsequent aggressiveness, remain unknown. In this study, we discovered that lactoferrin efficiently downregulates levels of ER-α, PR, and HER-2 in a proteasome-dependent manner in breast cancer cells, and it accounts for the loss of responsiveness to ER- or HER-2–targeted therapies. Furthermore, we found that lactoferrin increases migration and invasiveness of both non-TNBC and TNBC cell lines. We discovered that lactoferrin directly stimulates the transcription of endothelin-1 (ET-1), a secreted proinvasive polypeptide that acts through a specific receptor, ET(A)R, leading to secretion of the bioactive ET-1 peptide. Interestingly, a therapeutic ET-1 receptor-antagonist blocked lactoferrin-dependent motility and invasiveness of breast cancer cells. The physiologic significance of this pathway in the manifestation of TNBC phenotypes is revealed by elevated plasma and tissue lactoferrin and ET-1 levels in patients with TNBC compared with those in ER+ cases. These findings describe the first physiologically relevant polypeptide as a functional determinant in downregulating all three therapeutic receptors in breast cancer, which uses another secreted ET-1 system to confer invasiveness. Results presented in this article provide proof-of-principle evidence in support of the therapeutic effectiveness of ET-1 receptor antagonist to completely block the lactoferrin-induced motility and invasiveness of the TNBC as well as non-TNBC cells, and thus, open a remarkable opportunity to treat TNBC by targeting the ET-1 axis using an approved developmental drug.

IL-15

Track: *Regenerative Medicine*

THREE YEARS EXPERIENCE OF USING STEM CELLS THERAPY FOR HEART FAILURE AND PULMONARY HYPERTENSION IN CHILDREN

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Objective: Despite medical and surgical advances, heart failure and pulmonary hypertension are a frequent and life –threatening disorders. Stem cell therapy has shown significant positive results in animal studies. We can find quite a lot information about using stem cells to treat incurable diseases in adult population but whereas only few reports about treatment in childhood.

Method: The aim of this study is to assess the safety and efficacy of autologous bone marrow derived stem cells implantation intrapulmonary in children with severe pulmonary hypertension and intramyocardial implantation in children with dilated cardiomyopathy.

Result: Two children with Trisomy 21 and severe pulmonary hypertension due to uncorrected large ventricular septal defects had been admitted for the intrapulmonary bone marrow stem cell implantation. Both patients underwent radionuclide scintigraphy before and 6, 12 months after implantation. Latest results show improvement of lungs vascularization. Seven patients (from infant until teenager age) with dilated cardiomyopathy had been admitted for the intramyocardial transplantation of bone marrow derived progenitor cells. Every patient underwent follow-up every two months, we observed improvement of left ventricle systolic function by echocardiography and decrease of cardio thoracic index at chest X-ray, reduction of serum proBNP and decrease of the stage of heart failure.

Conclusion: Our findings show that the bone marrow derived progenitor stem cell transplantation is a safe method and might be used for the stabilization of the patient with pulmonary hypertension and heart failure to get the time for further treatment.

IL-169

Track: *Regenerative Medicine*

METHOD OF CHOICE FOR STEM CELL INTRAMYOCARDIAL IMPLANTATION IN CHILDREN

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Objective: Almost half of the children with symptomatic dilated cardiomyopathy receive a heart transplant or die within two years. Cardiac stem cell transplantation has become a promising method of treatment. Our decision is that in case of dilated cardiomyopathy in early age children, especially in critically sick children, intramyocardial stem cell implantation should be used transcutan under echocardiography guidance.

IL-141

Track: *Nutraceutical Drug Discovery & Therapy*

NEUROPROTECTIVE AND NEUROPRESERVATIVE EFFECTS OF NUTRACEUTICALS IN NEURONAL CULTURE SYSTEMS: POTENTIAL PREVENTIVE/THERAPEUTIC STRATEGIES IN ALZHEIMER'S DISEASE (AD)

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Alzheimer's disease (AD) accounts for the largest proportion of dementia cases in the elderly, and while five FDA-approved drugs for AD demonstrate significant improvements in cognition and global assessments, none cure the disease. Thus, new therapeutic strategies must be devised. Deposition of amyloid β (Aβ) peptides in the brain interstitial spaces followed by severe neuroinflammation is one of the key features of AD. Deposited Aβ can activate microglia, which in turn initiates inflammatory responses leading neuronal loss. Since AD has a complex and diverse etiopathogenesis, drugs interfering with a specific pathway of the disease pathology may not be suitable therapeutic candidates. This is reflected in the recent failures of several drugs in clinical trials. We hypothesize that drugs with pleiotropic properties, i.e. ability to modify several pathological cascades, can be potential preventive and/or therapeutic candidates for AD. In recent past, we have observed remarkable neuroprotective and neuropreservative properties of certain nutraceuticals both in neuronal culture and *in vivo*. Our interest to test synthesizable pure nutraceutical agents in AD models is based on the following fact: A large epidemiological study had depicted significant (~4.4-fold) less incidence of AD in Indian elderly when compared with a reference American populace (Chandra *et al.*, 2001). This finding indicates a strong environmental contribution in the development of AD, which may include dietary factors. We have observed that S-allyl-L-cysteine (SAC), an ingredient of garlic can preserve neurons from oxidative insults. SAC treatment in transgenic mice leads to preservation of pre-synaptic protein synaptophysin, which was observed significantly deficient in AD brain (Ray *et al.*, 2011a). In other studies, curcumin, a polyphenol found in turmeric root was found to preserve pre-synaptic protein SNAP25 in neuronal cells. We recently demonstrated that a nanoformulation of curcumin (NanoCurc™) has greater bioavailability than that of free curcumin and intra peritoneal administration of NanoCurc™ in mice resulted in robust brain concentrations of curcumin (Ray *et al.*, 2011b). We have also observed neuropreservatory properties of SAC, N-acetyl cysteine (NAC) and nanocurcumin in primary human fetal neuron cultures. Taken together, specific compounds obtained from several nutrients have displayed beneficial roles in preserving and protecting neurons from degeneration and can have potential therapeutic efficacy in AD. These results warrant further studies with these compounds in larger pre-clinical and clinical settings.

IL-18

Track: *Hot Topics in Drug Targets*

MICRORNA TO STUDY PHYSIOLOGICAL REGULATION OF GENE PRODUCTS IMPLICATED IN CNS DISORDERS: DISCOVERY OF NOVEL APP AND BACE-SPECIFIC MICRORNAS IMPORTANT FOR ALZHEIMER'S DISEASE

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The mammalian central nervous system (CNS) is a complex organ system, demanding an equally complex network of molecular pathways controlling the multitude of diverse, cellular activities.

Gene expression is a critical node at which regulatory control of molecular networks is executed. Thus, deciphering the various mechanisms employed in the physiological regulation of gene expression in the CNS is important for understanding the diseased state and for validating drug targets available for disease intervention. Our aim is to utilize the novel approach of studying the regulation of these gene products by microRNAs (miRNAs). miRNAs are an abundant class of small RNAs that mediate potent inhibitory effects on global gene expression. Recent advances in molecular methods allow us to study the contribution of these miRNAs to gene expression in CNS disorders, such as Alzheimer's disease (AD) (Long and Lahiri, *Experimental Neurology*, 2011). Aberrations in AD are believed to result, in part, from the over-production of amyloid-β peptide (Aβ), a product of Aβ precursor protein (APP). Expression studies suggest that dysregulation of proteins involved in Aβ production, such as APP and beta-secretase, or BACE1, may contribute to excess Aβ deposition. Elucidating how expression of these proteins is regulated will ultimately reveal new drug targets. Here we present data demonstrating miRNA-mediated regulation of APP and BACE1.

Recently, by using multiple bioinformatic tools and a series of functional studies in neuronal and glial cultures, we reported a specific microRNA (miR-101) regulates APP levels (Long and Lahiri, *Biochem. Biophys. Res. Commun.*, 2011). We and others have also identified additional set of miRNAs predicted to target the APP mRNA 3'-UTR and regulate APP levels (reviewed Long and Lahiri, *Curr. Med. Chem.*, 2011). Here we report the discovery of novel BACE1-specific miRNAs. First, we prepared a chimeric BACE1 3'-UTR reporter construct (10.1kb) by inserting the 3.9 kb BACE1 3'-UTR downstream of a reporter *Renilla* luciferase gene and then delivered the reporter construct along with several miRNAs predicted to target the BACE1 3'-UTR into human astroglial U373 cells. Several "hits" (e.g. miR-339-5p) resulted in reduced reporter expression. We further validated the reporter expression data for miR-339-5p by Western analysis of native BACE1 levels, which were significantly reduced following miR-339-5p delivery, with a potential in reducing toxic Aβ levels. Our results reveal a novel regulatory interaction between two important AD-related genes (APP and BACE1) and specific endogenously expressed miRNA species. These regulatory interactions are likely to serve as novel therapeutic targets and should enable the development of treatment strategies that may prove beneficial in the fight against AD.

This work is supported by grants from Alzheimer's Association and NIH to Dr. D.K. Lahiri.

IL-9

Track: *Regenerative Medicine*

CROSSTALK BETWEEN BONE MARROW-DERIVED STEM CELLS AND RENAL EPITHELIAL CELLS: EFFECTS ON CELL DEATH AND PROLIFERATION AS A PERSPECTIVE FOR CELL-BASED THERAPIES FOR NEPHROPATHIES

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During ischemic injury the proximal tubule cells are particularly affected, thus impairing kidney function. Bone marrow-derived cells (BMDC) and, more specifically, mesenchymal stem cells (MSC) can be mobilized to sites of injury where they play an important role in tissue recovery. In

the first part of this study we aimed to investigate the interaction between BMDC/MSC and renal tubular cells, by comparing their paracrine potential to protect and to stimulate cells proliferation. BMDC/MSC were obtained from murine and were co-cultured with LLC-PK1 renal epithelial cells using a porous membrane insert (4 μm pore diameter) to separate the two cell populations, thus allowing communication only by secreted factors. Renal cells proliferation was evaluated by counting viable cells or by PCNA immunofluorescence analysis. Cell death was determined by picnotic nuclei analysis, activated caspase-3 immunofluorescence or propidium iodide staining. Conditioned media were obtained by culturing BMDC/MSC for 72 h in serum free medium. The results showed that renal cells proliferated more rapidly when cultured with increasing concentrations of BMDC to produce a more effective stimulus. MSC are more effective in stimulating renal cells proliferation. The renoprotective effect was observed by the reduction of apoptosis in the presence of BMDSC/MSC. Again, increasing BMDC amounts decreases cell death, and MSC leads to a more marked reduction in cell death, indicating their higher paracrine potential. Proliferative and protective effects were not observed when renal cells were cultured with BMDC/MSC conditioned media. In the second part of the study, we have *in vivo* results (murine model) showing that the administration of BMDC to rats that had one urether obstructed leads to a reduction in fibrosis and collagen deposit within the renal tissue, as well as diminishes the infiltration of inflammatory cells. We also obtained significativa results concerning the bioactive lipid pattern in the renal tissue, where the BMDC treated group presents a higher content of sphingosine-1 phosphate and a fewer content of ceramide, been these lipids anti- and pro-apoptotic, respectively.

The results indicate that the crosstalk between BMDSC/MSC and renal cells is mediated by a paracrine mechanism that leads to protection and proliferation of remnant renal cells after injury thus suggesting a potential role for cell therapy in different nephropathies.

Keywords: Stem Cells, Renal Epithelial Cells, nephropathies.

IL-7

Track: *"Anti-Cancer Drug Discovery & Therapy"*

PERTURBATION OF THE TRANSLATOME BY THE ANTI-DIABETIC DRUG METFORMIN

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Metformin exhibits anti-proliferative and anti-neoplastic effects and its use is associated with reduced cancer incidence in type 2 diabetes patients.

The anti-proliferative effect of metformin has been linked to inhibition of mTORC1, but the molecular underpinnings of this phenomenon are still inadequately understood. Here we show that metformin primarily regulates gene expression at the level of mRNA translation, to an extent comparable to canonical mTOR inhibitors (rapamycin and PP242), and that its anti-proliferative activity is a consequence of selective translational suppression of mRNAs encoding cell cycle regulators via the mTORC1/4E-BP pathway. These findings reveal that metformin selectively modulates mRNA translation by suppressing mTORC1, suggesting applications in cancer prevention and treatment.

IL-198

FORMATION OF SILK FIBROIN NANOPARTICLES FOR CONTROLLED DRUG DELIVERY USING SUPERCRITICAL CO_2 TECHNIQUE

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The silk fibroin (SF) nanoparticles, around 50 nm in an average diameter and with a good spherical shape and narrow size distribution, were successfully prepared by a novel solution-enhanced dispersion by supercritical CO_2 (SEDS). The results of XRPD, TG-DSC, and FTIR analysis of the SF nanoparticles before and after ethanol treatment indicated conformation transition of SF nanoparticles from random coil to β-sheet form and thus water insolubility. Moreover, the relative non-toxicity of the silk fibroin nanoparticles after ethanol treatment shown by MTS assay indicated good

biocompatibility. A nonsteroidal anti-inflammatory drug, indomethacin (IDMC), was chosen as the model drug and was encapsulated in SF nanoparticles by the SEDS process. The resulting IDMC-SF nanoparticles, after ethanol treatment, possessed a theoretical average drug load of 20%, an actual drug load of 2.05%, and an encapsulation efficiency of 10.23%**.** *In vitro* IDMC release from the IDMC-SF nanoparticles after ethanol treatment showed a significantly sustained release over two days without a burst effect. In summary, the present investigation suggests that the silk fibroin nanoparticles prepared by the SEDS process can be used as a biocompatible carrier to deliver drugs. Also, the SEDS process provides a promising technique to fabricate drug-loaded polymer particles for the design of controlled drug delivery systems.

Keywords: Silk fibroin, nanoparticles, supercritical CO_2, indomethacin, drug delivery.

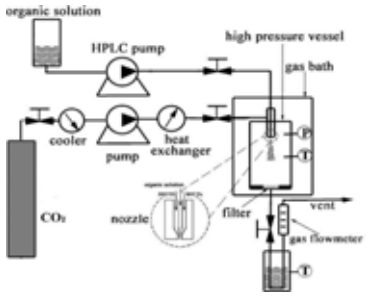

Fig. (1). Schematic diagram of the apparatus for the SEDS process.

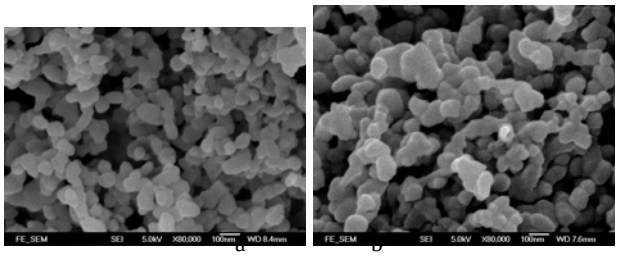

Fig. (2). FE-SEM images of SF nanoparticles (a) and IDMC-SF nanoparticles (b) prepared by the SEDS process.

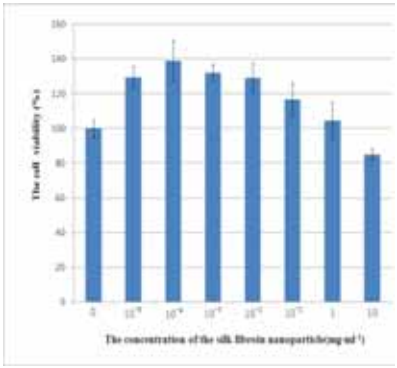

Fig. (3). The MTS cytotoxicity assay results of the human osteoblast-like SaOS-2 cells after 24 hours exposed with silk fibroin nanoparticles after ethanol treatment (untreated control set as 100%).

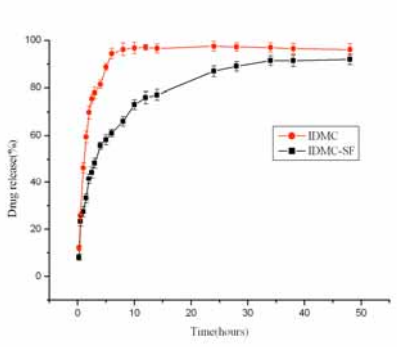

Fig. (4). Drug release curves of IDMC from IDMC-SF nanoparticles after ethanol treatment. Each point represents the mean ± standard deviation obtained from triplicates of the samples.

IL-172

Track: *Diabetes and Obesity Drug Discovery & Therapy*

SMALL MOLECULAR MODULATORS FOR GLP1 RECEPTOR

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Glucagon like-peptide-1 (GLP1) receptor holds several exciting promises for the treatments of diabetes and obesity, including increasing insulin secretion in the presence of high glucose, slowing gastric empty process, inhibiting glucagon release, reduce appetite and body weight, and stimulating B cell differentiation and replication. The agents that either activate GLP1 receptor directly (such as Byetta) or increase the half life of GLP1 peptide *in vivo* (such as Junuvia) have proven beneficial in the treatment of diabetes and potentially obesity. Small molecular agonist and/or sensitizer are a new oral approach to fully realize GLP1 receptor activities *in vivo*. These agents have been shown to produce

significant glucose, lipid and/or body weight control in animal models without the many liabilities that are associated with other oral therapies, including weight gain, edema, and hypoglycemia. Accordingly, small molecular modulators have excellent potential for use in both monotherapy and combination with established agents, such as Byetta and Junuvia. Extensive efforts in many other laboratories have failed in the identification of a drug-like oral available small molecular agonist or sensitizer for GLP1 receptor. Our small molecular agonist or sensitizer may hold the 1st/best in the class opportunity of GLP1 receptor for diabetes and obesity treatment, representing an innovative oral therapy for treatments of these diseases to achieve desired therapeutics index.

IL-56

Track: *Drug Metabolism*

DISCOVERY OF CC-930, AN ORALLY ACTIVE ANTI-FIBROTIC JNK INHIBITOR

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Improving the physico-chemical properties as well as increasing the potency and selectivity of a series of compounds with adequate rat plasma exposure, led to the identification of JNK inhibitors. Differentiation based on PK profiles in multiple species as well as activity in a chronic efficacy model led to the identification of CC-930 as a development candidate, which is currently in Phase II clinical trial for idiopathic pulmonary fibrosis. This process as well as some metabolism data will be discussed.

IL-49

Track: *Academic CRO/Industrial collaborations in drug discovery*

HOW MUCH DOES IT REALLY COST TO DEVELOP A DRUG FOR RARE AND NEGLECTED DISEASES?

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Pharmaceutical innovation is in the midst of a well-publicized crisis that has caused 8 large drug firms to disappear since 2000, and has started to shrink the revenues of the remaining ones. The R&D spending per new drug at the largest 10 companies now averages $9 billion, which in turn is pushing the price of new drugs to tens of thousands of dollars per course of treatment. Under these conditions, one would expect the development of new medicines for rare and neglected diseases to dry up since the market for such drugs is small and offers poor returns. Yet, a survey of the last 10 years shows that the production of such drugs is on a steady but increasing trend. Almost 30 NMEs have been approved for rare and neglected diseases since 2002, the majority in the last 5 years. Surprisingly, nearly all these drugs have been sponsored by small companies or non-profit organizations with limited resources. Since most of these organizations are either public companies or voluntarily disclose their accounts, it is possible to analyze their spending and estimate how they spent to bring their drugs to market. The resulting data can then be compared to big pharma's to better understand the roots of the innovation crisis and whether big pharma's model can be fixed or must be replaced by something new such as the model now being perfected on rare and neglected diseases.

IL-91

Track: *Academic CRO/Industrial collaborations in drug discovery*

TRANSLATIONAL RESEARCH BASED ON IMAGING BASED DRUG DISCOVERY

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Institut Pasteur Korea is a translational research institute bridging basic research and therapy development. Starting with the visualizing critical disease steps in living cells, IP-Ks approach exploits advanced imaging to study the effect of chemical compounds on cell based disease models.

The consequent implementation of imaging technology allows IP-K to approach biotechnological systems from an IT angle, allowing to find effective drug leads in both infectious and chronic disease scenarios. It transforms the integrated cell based disease model into the actual target, with compounds addressing any of the cellular factors involved. The imaging driven approach identifies drugs that are effective and at the same time do not reveal cellular toxicity.

Where previously targets would be the starting point for the development of drugs, it is now the effective compound, which allows to us to identify new targets in a second step. Starting target-free, this procedure then effectively reverses the drug discovery process. Genome-wide screening of visual arrays in the presence of effective compounds allows IP-K to zoom in on novel targets within days. Our successful screening campaigns on the infectious and chronic disease models demonstrate that the recent, Pharma-wide trend towards target free approaches can be harnessed into effective routine procedures.

Examples for IP-K's drug discovery in infectious and chronic diseases will be presented as well as the automated target ID process.

IL-51

Track: *Hot Topics in HIV Research*

DEVELOPMENT OF MODIFIED NUCLEOSIDES THAT HAVE EXTREMELY HIGH ANTI-HIV ACTIVITY AND LOW TOXICITY AND PREVENT THE EMERGENCE OF RESISTANT HIV MUTANTS, AND A PROPOSED STRUCTURE OF MODIFIED NUCLEOSIDE EXPECTED TO HAVE HIGH ANTIVIRAL ACTIVITY AND LOW TOXICITY

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An idea to use 4'-*C*-substituted-2'-deoxynucleoside derivatives was propos ed based on working hypotheses to solve the problems of the existing AIDS chemotherapy. Subsequent studies have successfully proved the validity of the idea and resulted in the development of 2'-deoxy-4'- *C*-ethynyl-2-fluoroadenosine and 2'-deoxy-4'-*C*-ethynyl-2-chloroadenosine which have extremely high activity against all HIV and prevent the emergence of resistant HIV mutants. During the study, it was found that substrate selectivity is different between viral nucleic acid polymerases and human nucleic acid polymerases.

A general structure of modified nucleoside expected to have high antiviral activity and low toxicity is proposed based on the finding.

IL-166

Track: *Recent Advances in Patient Treatment*

USERS AS DEVELOPERS AND ENTREPRENEURS OF MEDICAL TREATMENTS/ DEVICES: THE CASE OF PATIENTS AND THEIR FAMILIES AND FRIENDS

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The health care industry has experienced a proliferation of innovations aimed at enhancing life expectancy, quality of life, diagnostic and treatment options. Previous research has shown that users themselves innovate with respect to service they self provide. In this study, we look at sources of health care innovations, in particular the role of users in the development of those innovations. We build our study upon previous work by Oliveira, von Hippel and DeMonaco (2011) and Oliveira (2012) and will empirically explore the role of patients and their families and friends (i.e. users) in developing new medical treatments and devices for themselves. We focus on chronic diseases, including respiratory

diseases (Cystic Fibrosis, Asthma and Sleep Apnea), Cancer, Diabetes and medical devices and treatment for general purposes. For each of these chronic disease we developed a sample of treatments and devices and show that patients with those diseases (or their families and friends) have developed a significant proportion of treatments and devices. Despite some sampling limitations, we found that the majority (54%) of new medical treatment and devices for those disease were developed by patients or their families and friends. Moreover, 53% of such patients who are users innovations have become entrepreneurs and created their own firms to produce their inventions.

Our empirical findings have important policy and managerial implications for health care management and entrepreneurship.

IL-130

Track: *CNS Drug Discovery & Therapy*

TFP5: A PEPTIDE DERIVED FROM THE CDK5 REGULATOR P35, CROSSES BLOOD BRAIN BARRIER AND RESCUES PHENOTYPES OF AD MODEL MICE

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Cyclin-dependent kinase 5 (Cdk5), unlike its other cyclin-dependent family members is unique as it is activated only by neuron-specific proteins p35 and p39. The activity of Cdk5/p35 is tightly regulated in the developing and mature nervous system but under neuronal stress, p35 is cleaved by calpain, a calcium activated protease into p25 and p10 fragments. Truncated p25 forms a more stable Cdk5/p25 complex which has higher activity and longer half-life that aberrantally hyperphosphorylates cytoskeletal proteins (tau and neurofilament) inducing cell death. Hyperphosphorylation of tau leads to the formation of the neurofibrillary tangles (NFT) which are one of the hall marks of Alzheimer's disease (AD) along with senile amyloid-beta plaques and axonal transport of aberrantally hyperphosphorylated neurofilament proteins is inhibited induces neuronal restrained. It has also been reported that AD brains have higher levels of p25 and Cdk5 activity, suggesting that the Cdk5/p25 complex may be an ideal therapeutic target for AD. The current therapeutic approach lacks specificity as it is based on the compounds resembling roscovitine (a kinase inhibitor) which competes with ATP binding site on Cdk5 and other kinases and therefore not only inhibits Cdk5/p25 activity but also Cdk5/p35 activity as well as other kinases. In order to search for a specific inhibitor our approach based on the crystal structure of Cdk5/p25, resulted in several small truncated molecules of p25 that inhibited Cdk5 phosphorylation *in vitro*. Out of many, one of the truncated fragments of p25, a 24 amino acid residue (called p5) inhibited Cdk5/p25 activity more effectively than other larger forms. In addition *in situ* p5 specifically inhibited Cdk5/p25 deregulated activity. Encouraged by these studies, we modified P5 as TFP5 and studied its effect in AD model mice. It is found that TFP5 crosses the blood brain barrier (BBB) and rescues the AD pathology in the AD model mice. The rescue effect is also seen in various behavior-tests for spatial memory loss and motor deficit indicating that TFP5 could be a good therapeutic candidate for AD.

IL-46

Track: *Cardiovascular Drug Discovery & Therapy*

CYTOKINES, MACROPHAGES AND ATHEROSCLEROSIS

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Atherosclerosis is a major cause of morbidity and mortality worldwide. Macrophages play a crucial role in atherosclerosis by modifying lipoproteins, accumulating intracellular lipids, amplifying the inflammatory response and remodeling the extracellular matrix. Regulation of macrophage activities therefore represents a promising strategy for the prevention and treatment of atherosclerosis. Because atherosclerosis is an inflammatory disorder orchestrated by cytokines, the action of these on macrophage function and properties will have a major impact on the initiation and progression of this disease. We have therefore been investigating the action of several cytokines on macrophage lipid homeostasis, particularly the uptake of modified lipoproteins, intracellular metabolism of cholesterol and the efflux of

this sterol along with the inflammatory response. These studies have provided novel insight into the molecular mechanisms underlying the regulation of macrophage cholesterol homeostasis and the inflammatory response by interferon-gamma, transforming growth factor-beta, tumour necrosis factor-like protein 1A and interleukin-33. In addition, we have identified the potential mechanisms underlying the anti-inflammatory actions of lipid lowering drugs such as statins and nuclear receptor agonists. These findings will be presented in relation to current and future approaches that target cytokine action and macrophage function for therapeutic intervention of atherosclerosis.

IL-102

ATHEROSCLEROSIS: MOLECULAR MECHANISMS, THERAPEUTIC TARGETS AND TRANSLATIONAL CHALLENGES

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Atherosclerosis is the primary cause of heart disease and stroke and thereby a leading cause of morbidity and mortality worldwide. The underlying pathogenesis of atherosclerosis involves an imbalance in both lipid homeostasis and the immune response resulting in chronic inflammation of the arterial wall and the development of complex plaques. Although the discovery of cholesterol lowering drugs (statins) have had a marked effect in reducing mortality from cardiovascular disease, several studies have highlighted the residual risk, particularly in high-risk patients, thereby substantiating the need to identify more therapeutic targets and avenues. Research in the last decade, particularly using mouse model systems, have significantly improved our understanding of the pathogenesis of this disease and identified a number of potential therapeutic targets. Such targets include key enzymes involved in the modification of low-density lipoprotein, factors required for both the recruitment of immune cells to atherosclerotic plaques and controlling the inflammatory response, proteins and microRNAs that are critical for regulating lipid homeostasis and proteases that destabilize plaques and cause their rupture. However, major gaps remain in terms of translation to clinic. Recently identified molecular mechanisms underlying atherosclerosis, translational development and clinical strategies will be presented.

IL-104

Track: *Innovative Drug Discovery and Nanotechnology*

PEPTIDE DESIGN AND SELF·ASSEMBLY FOR BIOMEDICAL APPLICATIONS

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Designing peptide self·assemblies provides an efficient strategy for linking molecular structure and function. Steady interest to the strategy extends beyond basic research and prompts the emergence of novel biomaterials with unprecedented properties defined at the nanoscale.1 Synthetic designs derived from biological nanoscale assemblies underpin various applications of biomedical importance. Examples may include gene delivery systems,2 fibrillar microscopic structures for tissue repair3 and responsive antimicrobial agents4. A key factor in all such designs is their structural and functional relevance to native macromolecular assemblies, be these viruses, extracellular matrices or host defence systems. Our ability to construct such materials at will not only advances our understanding of functional self·assembly but also facilitates in developing new efficient technologies.

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IL-128

Track: *Hot Topics in HIV Research*

STRUCTURAL BIOLOGY OF HIV-HOST INTERACTIONS: BASIS FOR THERAPEUTIC INTERVENTION

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Human immunodeficiency virus type 1 (HIV-1) encodes a polypeptide called Gag that is capable of forming virus-like particles *in vitro* in the absence of other cellular or viral constituents. HIV-1 Gag is transported to the plasma membrane (PM) for assembly through discrete interactions with cellular transport pathways. We employ a set of biochemical, biophysical, structural biology and *in vivo* tools to identify key protein-protein and protein-lipid interactions involved in the molecular mechanisms governing HIV-1 Gag intracellular trafficking and subsequent assembly on the PM. In this presentation, we provide structural details on how Gag interacts with calmodulin, a highly-conserved calcium-binding protein expressed in all eukaryotic cells. We also provide structural basis for Gag interactions with phosphatidylinositol-4,5-bisphosphate, a cellular marker localized on the PM and proved to be critical for HIV assembly. We identified small molecule inhibitors that are able to disrupt Gag interactions with CaM and phosphatidylinositol-4,5-bisphosphate. Remarkably, these inhibitors were able to inhibit HIV production by up to ~80%. Our studies not only provide detailed mechanisms of how HIV proteins interact with the host but also aid in the development of new therapeutic strategies for treatment of emerging multi-drug resistant strains of HIV.

This work is supported by the US National Institutes of Health (1R01AI087101).

IL-114

Track: *Structural Biology*

INTRODUCING COMPUTATIONAL STRUCTURAL SPECTROSCOPY OF GLOBULAR PROTEINS

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A new Monte Carlo method, based on the potential energy landscape of a globular protein, will be described that characterizes both static and dynamic protein void structures. For a specific protein, this algorithm produces a characteristic "spectrum" for the cavity size distribution as well as a size spectrum of percolating and non-percolating paths through the protein. By using molecular dynamics, the algorithm can be applied to a thermally fluctuating protein, and as expected, characteristic peaks in the spectrum broaden. Bottle necks in percolation paths can be readily indentified in static structures, and when the protein is allowed to thermally fluctuate, these bottlenecks can open and close with time. Implications of this new structural tool for characterizing protein function and drug design will be discussed.

Keywords: Globular protein, Spectrum.

IL-85

Track: *Academic CRO/Industrial collaborations in drug discovery*

STAYING COMPETITIVE: CREATING AND FOSTERING INNOVATION IN THE WORK PLACE

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Staying competitive as an organization is becoming more and more challenging because of growth in globalization of markets, global competition for talent, exponential growth in information and knowledge, and the lightning speed of development of new technologies. How do we keep our organizations competitive in this environment? This presentation will examine the importance of innovation and finding new ways to create value in a global marketplace and fostering innovation in an organization.

IL-37

Track: *Anti-cancer*

TARGETING PI3-K/AkT/CK2/PTEN CASCADE AND TUMOR MICROENVIRONMENT AS A NOVEL APPROACH FOR CANCER THERAPY

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There is accumulating evidence that tumor microenvironment is critically involved in supporting survival of tumor cells. We applied an *ex-vivo* microenvironment model to explore the role of PI3-K/Akt/PTEN cascade in disease progression and as a potential therapeutic target in chronic lymphocytic leukemia (CLL).

Primary stromal cells from bone marrow induced the activation of PI3-K/Akt cascade and inhibited apoptosis of CLL cells. Pan- and isoform-selective PI3-K inhibitors and specific siRNA counteracted the anti-apoptotic effect of the stromal cells. Induction of apoptosis was associated with a decrease in PIP3, dephosphorylation of Akt1 and PTEN. Freshly isolated CLL cells showed significantly higher levels of phosphorylated Akt1, PTEN and CK2 than healthy person samples. CK2 inhibitors decreased phosphorylation of PTEN and Akt, induced apoptosis in CLL cells and enhanced the response to fludarabine.

The data demonstrate the central role of the microenvironment and PI3-K/Akt/PTEN cascade in the pathogenesis of CLL. They also provide a proof of concept on the therapeutic value of targeting PI3-K/Akt/CK2 in combination with the recovery of PTEN tumor suppressor activity as a novel concept for treatment of CLL and probably other human neoplasias. Clinical protocols have been initiated with the orally available PI3-K inhibitor NVP-BEZ235.

IL-137

Track: *"Anti-Cancer Drug Discovery & Therapy"*

SELF-SACRIFICED PEG-SHEDDABLE MICELLES BASED ON DISULFIDE BOND CONJUGATION FOR GLUTATHIONE-MEDIATED INTRACELLULAR DRUG DELIVERY

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Micelles with unique “self-sacrificed” property are reported for rapid intracellular doxorubicin (DOX) release in response to glutathione at the cellular level (GSH). The micelles are developed with the PEG-sheddable shell and poly (ε-benzyloxycarbonyl-L-lysine) core based on disulfide bond conjugation. These micelles will disassemble (or sacrifice) within 4 h in response to 10 mM GSH due to detachment of PEG shells, evidenced by size alterations, a phenomenon described as “self-sacrificed micelles”. Furthermore, the *in vitro* release of DOX-loading micelles in the presence of GSH is 3-5 times faster than the control. CLSM observations demonstrate that the DOX-loaded PEG-detachable micelles can be internalized into human breast cancer cells (MCF-7) and exhibit much faster release of DOX both in cytoplasm and cell nuclei under GSH reduction environment compared to the non-reduction environment. Importantly, as GSH level increases, the inhibition activity for cell proliferation is intensified, due to faster intracellular release of DOX.

Keywords: self-sacrificed, PEG-sheddable micelles, disulfide bond conjugation, glutathione-sensitive, drug delivery.

IL-59

Track: *Innovative Drug Discovery and Nanotechnology*

PROTEOMIC PROFILING OF THE DYNAMIC NANOPARTICLE-SERUM PROTEIN CORONA - IMPLICATIONS FOR BIOMEDICAL APPLICATIONS

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For medical applications, nanoparticles (NPs) are frequently administered parenterally. However, blood proteins rapidly associate with NPs, leading to a dynamic protein ''corona'' defining the biological identity of the particle, influencing biodistribution, toxicity and delivery to intended target sites. Thus, a rational design and effective application of NPs requires detailed knowledge of the particle-associated proteins, a fundamental prerequisite for nanomedicine and nanotoxicology.

Using label-free liquid chromatography mass spectrometry, we present the qualitative and quantitative composition of the human blood plasma protein corona on polymer and amorphous silica NPs differing in size and surface functionalization. We identified and bioinformatically characterized >150 different proteins in the respective NP coronas. Notably, the protein fingerprints were not only significantly influenced by the physico-chemical NP characteristics but dynamically varied also over time (1 – 120 min). We report a comprehensive particle- and time-dependent dynamic enrichment of bioreactive serum proteins such as lipoproteins, proteins involved in coagulation or the complement pathway.

Our results will contribute to the rational design of nanoparticles for drug delivery, as imaging agents, or for diagnostic purposes, and will have deep implications for the interpretation of (pre)clinical experimental outcomes.

IL-12

Track: *Hot Topics in Drug Targets*

HUMAN LIPOXYGENASE: STRUCTURE, FUNCTION, RELEVANCE TO HUMAN DISEASES AND CHALLENGES IN DRUG DEVELOPMENT

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Lipoxygenases (LOXs) are the enzymes participating in the metabolism of the polyunsaturated fatty acids (PUFA) and catalyzing their oxidation to a variety of eicosanoids, which as the secondary signal transducers have a major impact on human homeostasis. They are involved in many diseases such as all ailments with an inflammatory component, cancers, cardiovascular and kidney diseases, neurodegenerative disorders and metabolic syndrome. Using variety of experimental procedures we have found that human platelet 12-lipoxygenase is biologically active as oligomer (minimum dimer) with apo and active molecules. The monomer might have 2 regulatory domains ~110 residues each at the opposite ends, and (~440) catalytic domain in between. These domains show independent movement responsible for dimer's flexibility that may impact an orchestrated allosteric mechanism. Naturally occurring SNP R261Q shows altered kinetics but unaffected specificity. Utilizing specific antibodies we have established that human lipoxygenases can coexist in tissue but may exhibit opposite trend of expression for example in cancerous tissue during the course of disease (5-, 12- vs. 15-LOX-1). Therefore drug development in addition to finding the specific inhibitors would require the detailed knowledge about each enzyme behavior, mechanism of action, the structure-function relationship and cohabitation with other oxygenases.

IL-154

Track: *Drug Discovery in Preclinical Research*

NEXTGEN VENOMICS FOR PEPTIDE DRUG DISCOVERY AND LEAD OPTIMISATION USING MELUSINE®: NATURAL, SYNTHETIC AND VIRTUAL VENOM LIBRARIES

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Venoms are made of hundreds of peptides optimised by Nature as highly selective and potent bioactives that already led five drugs to market. We pioneered *Venomics* drug discovery and lead optimisation strategies integrating bioactivity-guided identification (*Melusine*®, unique collections of HTS-ready pre-fractionated venoms) and structure-driven characterization (venom peptidomics & proteomics, venom glands transcriptomics and genomics) coupled to biocomputing-assisted analysis (proprietary databases and bioinformatic tools).

We now present our ***NextGen Venomics platform*** to exploit rare samples in an unprecedented manner through a combination of natural, synthetic and virtual venom libraries focusing on:

- Massive venom gland mRNA sequencing and assembly using NextGen strategies
- Bioinformatics tools to extract relevant sequences and produce the ***first virtual venoms***
- Original software to streamline the transcriptomics-assisted deconvolution process
- An integrated *in silico* platform to screen virtual venoms for analogues of hits and leads
- Large-scale multiplex synthesis of natural peptides as ***first synthetic venoms libraries***

We are convinced that our innovative ***NextGen Venomics strategies*** will pave the way to the future developments of venom-based drug discovery.

IL-68

Track: *Drug Metabolism*

EFFICIENT TESTING FOR METABOLISM-BASED DDIS IN DRUG DISCOVERY AND THE IMPLICATIONS FOR CONCORDANCE WITH MANDATED ASSAY METHODOLOGIES USED IN DRUG DEVELOPMENT

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Drug-drug interaction screening in early ADME programs consists primarily of cytochrome P450 inhibition assays. In discovery, breadth of testing is dictated by a number of factors including strategy, therapeutic area, risk tolerance and available resources relative to demand, all of which are dependent on internal company factors. Moreover, a variety of assay methodologies are available using whole cells, liver extracts or recombinant enzymes together with either drug substrates, fluorometric substrates or luminometric substrates. These latter two substrate classes can be more resource efficient. In development, several assays are mandated by regulatory agencies (e.g. USFDA, EMEA, etc). These typically include determination of inhibition constants with 6 cytochrome P450 enzymes (CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4) with specified drug substrates in human liver microsomes as well as assessment of enzyme inactivation after preincubating with NADPH prior to addition of the drug probe substrates. A variety of factors can affect the quantitative inhibition potency results obtained from these assays. These include substrate-dependent inhibition (e.g. differences in binding interaction, lack of absolute specificity of the probe substrate), futile binding of the inhibitor, inhibitor depletion and substrate depletion. Therefore, differences in assay methodology can result in inconsistencies or "surprises." This presentation will focus on sound and consistent practices for the implementation of efficient cytochrome P450 inhibition assays in both discovery and development.

IL-74

Track: *Drug Metabolism*

AN IGF-1R INHIBITOR INDUCES CYP3A4 EXPRESSION THROUGH A PXR-INDEPENDENT, NON-CANONICAL CAR-RELATED MECHANISM

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Inhibition of the insulin-like growth factor-1 receptor (IGF-1R) sign aling represents an attractive therapeutic strategy for cancer treatment. A first generation IGF-1R inhibitor BMS-536924, however, was associated with potent CYP3A4 induction mediated by pregnane X receptor (PXR) transactivation. Structure activity-based modification led to the synthesis of BMS-665351 with no PXR activity while maintaining its ability to inhibit IGF-1R. However, BMS-665351 significantly induces CYP3A4 expression in human primary hepatocytes (HPHs). Here, we report a novel non-classical constitutive androstane receptor (CAR)-related pathway of BMS-665351-mediated CYP3A4 induction. BMS-665351 treatment resulted in significant induction of CYP3A4 in HPHs and HepG2 cells, but failed to activate either PXR or CAR in cell-based reporter assays. Moreover, BMS-665351 at concentrations that induce CYP3A4 expression was unable to translocate human CAR from the cytoplasm to the nucleus of HPHs. Nevertheless, real-time PCR analysis demonstrated that BMS-665351 significantly enhanced the expression of CYP3A4 in CAR- but not PXR-transfected HepG2 and Huh7 cells. Notably, BMS-665351 selectively induced the expression of CAR but not PXR in all tested cell systems. Synergistic induction of CYP3A4 was observed in HPHs co-treated with BMS-665351 and prototypical activators of CAR but not PXR. In summary, our results indicate that BMS-665351-mediated induction of CYP3A4 is CAR-dependent, but BMS-665351 itself is not a typical activator of either CAR or PXR, rather it functions as a selective inducer of CAR expression and increases CYP3A4 through a non-canonical CAR-related mechanism.

IL-29

Track: *Hot Topics in Natural Products*

CHRYSIN PROTECTS AGAINST CISPLATIN-INDUCED COLON TOXICITY VIA AMELIORATION OF OXIDATIVE STRESS AND APOPTOSIS: PROBABLE ROLE OF p38MAPK AND p53

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Cisplatin, an antineoplastic drug, widely used as a foremost therap y against numerous forms of cancer but it has pronounced adverse effects *viz.*, nephrotoxicity, ototoxicity etc. CDDP-induced emesis and diarrhoea are also marked toxicities that may be due to intestinal injury. Chrysin (5,7-dihydroxyflavone), a natural flavone commonly found in many plants possesses multiple biological activities, such as antioxidant, anti-inflammatory and anti-cancer effects. In the present study, we investigated the protective effect of chrysin against CDDP-induced colon toxicity. The plausible mechanism of CDDP-induced colon toxicity and damage include oxidative stress, activation of p38MAPK and p53, and colonic epithelial cells apoptosis via upregulating the expression of Bak and cleaved caspase-3. Chrysin was administered to Wistar rats once daily for 14 consecutive days at the doses of 25 and 50mg/kg body weight orally in corn oil. On day 14, a single intraperitoneal injection of cisplatin was given at the dose of 7.5mg/kg body weight and animals were sacrificed after 24

hour of cisplatin injection. Chrysin ameliorated CDDP-induced lipid peroxidation, xanthine oxidase activity, glutathione depletion, decrease in antioxidant (catalase, glutathione reductase, glutathione peroxidase and glucose-6 phosphate dehydrogenase) and phase-II detoxifying (glutathione-s-transferase and quinone reductase) enzymes activities. Chrysin also attenuated goblet cell disintegration, expression of phospho-p38MAPK and p53, and apoptotic tissue damage which were induced by CDDP. Histological findings further supported the protective effects of chrysin against CDDP-induced colonic damage. The results of the present study suggest that the protective effect of chrysin against CDDP-induced colon toxicity was related with attenuation of oxidative stress, activation of p38MAPK and p53, and apoptotic tissue damage.

Keywords: Cisplatin, Colon toxicity, Oxidative stress, Apoptosis, p38MAPK, Bak.

IL-10

Track: *CNS Drug Discovery & Therapy*

CHEMICAL CHAPERONE THERAPY FOR NEURONOPATHIC LYSOSOMAL DISEASES

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Chemical chaperone therapy is a new molecular therapeutic approach to lysosomal diseases based on a paradoxical molecular phenomenon involving lysosomal enzyme protein and its competitive inhibitor as intracellular enhancer (chaperone). The misfolded mutant enzyme protein is stabilized by formation of a molecular complex with its substrate analogue, and transported safely to the lysosome. The complex is automatically dissociated in the lysosome, the free mutant protein normally folded remains stable, and the enzyme activity is expressed. The small chaperone molecule is delivered to the brain tissue through the blood-brain barrier. G_{M1}-gangliosidosis, a lysosomal disease, has been our major target by a novel galactose analogue, N-octyl-4-epi-β-valienamine (NOEV), for restoration of the mutant enzyme β-galactosidase in the central nervous system. After oral administration NOEV was delivered to the brain in the genetically engineered G_{M1}-gangliosidosis model mouse. The enzyme activity was elevated, and substrate storage was reduced. Neurological deterioration was completely arrested, and survival time was significantly prolonged. Recently another chaperone MTD118, a bicyclic azasugar, has become available. The two chaperones exhibited different (partly complementary) chaperone spectra. We anticipate that the combination of NOEV and MTD118 will be applicable to more than two-thirds of the patients with β-galactosidase deficiency disorders caused by missense gene mutations. Computational analysis indicated that the chaperone-enzyme complex is less stable with higher binding free energy at pH 5 than at pH 7, thus allowing spontaneous dissociation in the lysosome. Each chaperone has its own molecular specificity toward the enzyme counterpart. Restoration of the enzyme activity depends on the steric conformation of the chaperone structure that determines stability of the chaperone-enzyme complex. Theoretically this principle can be applied to other lysosomal diseases, if a specific chaperone compound becomes available for each target enzyme. Furthermore, many other neurogenetic diseases caused by misfolding of mutant proteins will be considered for chemical chaperone therapy.

IL-149

Track: *Drug Discovery in Preclinical Research and sub-track b) Hit to Lead and Lead Optimization*

NOVEL BRAIN-CHIP TECHNOLOGY PROVIDES BREAKTHROUGH FOR HIGH TO MID-THROUGHPUT DRUG SCREENING OF NEURONAL AND CARDIAC CELLS

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Planar patch-clamp chips have been developed to screen drugs targeted at ion channels embedded in neuronal and cardiac cells. However, the available planar patch-clamp chip approach is limited only to suspended cell lines transfected with ion channels – thus limiting their physiological and pathological utility. We report here for the first time, on the development of planar patch-clamp chips suitable for recording ion channel activity from cultured neurons placed either at single- (silicon: Si chips) or dual-sites (polyimide: PI chip). This approach allows us not only to monitor ion channel activities underlying intrinsic membrane properties but also provides access to synaptic currents between the paired cells. We successfully recorded evoked post-synaptic potentials (EPSPs) and currents (EPSCs), and synaptic potentiation that forms the basis for learning and memory in the nervous system. Simultaneous, dual-site chip recordings were obtained over days from synaptic pairs, and dedicated cytoplasmic perfusion of individual neurons *via* on-chip subterranean microfluidics was possible without disrupting the whole-cell configuration. Recordings of synaptically connected neurons on a patch-clamp chip have tremendous value as a model to investigate synaptic function, and also as an advance drug development too for diseases such as Epilepsy, Parkinson, Alzheimer's and mental disorders.

IL-73

Track: *Academic CRO/Industrial collaborations in drug discovery*

DISCOVER EU2P: THE FIRST EUROPEAN PROGRAMME IN PHARMACOVIGILANCE AND PHARMACOEPIDEMIOLOGY

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The European Commission through the Innovative Medicines Initiative wants to revolutionise drug research by providing highly-skilled medicines stakeholders. For the first time in the history of pharmaceutical research, big companies are working with universities, patients and regulators, to share their knowledge and expertise for a common education and training objective.

Eu2P is the first European public-private education and training initiative to improve citizens' healthcare.

Eu2P gathers seven Universities, the European and French Medicines Agencies and fifteen Pharmaceutical Companies.

Eu2P fosters the knowledge and understanding of medicines use for healthcare professionals, students and non-specialists. This academic programme especially provides training in medicines benefit assessment, regulatory aspects, risk quantification, public health and risk communication.

Eu2P offers valuable diplomas: one Master, one PhD and Certificates jointly awarded by all public and private partners. Emphasis is put on hands-on training to maximise post-training employment opportunities.

By joining Eu2P, all stakeholders choose e-learning to learn with experts in pharmacovigilance and pharmacoepidemiology at anytime, anyplace.

IL-132

Track: *Inflammation and Immunology*

ZAPS AS A BOOSTER OF INNATE IMMUNE SIGNALING FOR ANTIVIRAL DEFENSE AGAINST INFLUENZA VIRUS INFECTION

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Pathogen invasion is sensed by pattern recognition receptors (PRRs) of the innate immune system through their recognition of pathogen-associated molecular patterns (PAMPs). This triggers innate immunity and the subsequent activation of adaptive immunity for efficient eradication of pathogens. During viral infection, viral nucleic acids serve as a major pathogen–associated molecular pattern, which is targeted by nucleic acid sensors, a subgroup of host PRRs, such as TLRs (Toll-like receptors), RLRs (RIG-I-like receptors), ALRs (AIM2-like receptors), and others. Among these, RIG-I has been found to be a key cytosolic PRR for the detection of RNA derived from various RNA viruses such as influenza virus, measles virus and hepatitis C virus, most of which are closely related to the pathogenesis of infectious diseases in human. RIG-I activation by the sensing of viral RNAs initiates the downstream gene induction program, which leads to the productions of type I interferons (IFNs) and proinflammatory cytokines/chemokines that are crucial for activating innate immune responses to those viruses. Our group has recently identified the shorter isoform of poly(ADP-ribose) polymerase (PARP)-13 "ZAPS" as a potent stimulator of RIG-I-mediated signaling for antiviral responses. ZAPS was found to interact with RIG-I in a ligand-dependent manner to enhance RIG-I activation, which results in robust activation of its essential downstream transcriptional factors, IRF-3 and NF-kappaB. We also showed that ZAPS is a key factor to enhance antiviral innate defense during influenza virus infection. In relation to this finding, we further found an intriguing interrelationship between ZAPS and an influenza viral protein. Our data suggest a novel ZAPS-related mechanism for the innate immune evasion by influenza virus. Thus, ZAPS plays important roles as an antiviral factor in the innate immune system, and our data suggests that ZAPS might be a useful therapeutic target for control of influenza virus infection.

IL-57

ZINC FINGER PROTEIN DESIGNED TO TARGET 2-LTR-CIRCLE JUNCTIONS INTERFERES WITH HIV INTEGRATION

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Integration of the HIV-1 genome into the host chromosome is a vital step in the HIV life cycle. A highly conserved dinucleotide sequence CA immediately upstream of the cleavage site is crucial for integrase (IN) activity. As this viral enzyme plays an important role in the early event of replication cycle of HIV-1, interference with the IN substrate has become an attractive strategy for therapeutic intervention. Recently, we demonstrated that a designed zinc finger protein (ZFP) fused to green fluorescent protein (GFP) targets the 2-long terminal repeat-circle junctions of HIV-1 DNA. This ZFP, 2LTRZFP-GFP, specifically binds to target DNA with nanomolar affinity. In this study, we showed that 2LTRZFP-GFP stably transduced into 293T cells interfered with the expression of VSV-G pseudotyped lentiviral red fluorescent protein (RFP) as shown by suppression of RFP expression. In addition, we used a third-generation lentiviral vector and pCEP4 expression vector to deliver the 2LTRZFP-GFP transgene into human T-lymphocytic cells and selected the stable lines for a long-term expression. HIV-1 integration and replication was inhibited in the stable line as measured by p24 antigen assay. The results were confirmed with *Alu-gag* real-time PCR for integration interferences. The 2LTRZFP-GFP limited viral integration upon intracellular immunization and has potential for use in HIV gene therapy in the future.

IL-3

Track: *Regenerative Medicine*

EVALUATION OF ADVANCED NANOMATERIAL TO CONTROLLED RELEASE OF PHOTOACTIVE COMPOUNDS UNDER SKIN DERMAL EQUIVALENT TO IMPROVE WOUND HEALING AND BURNING TREATMENT OF SKIN DISORDER IN TISSUE ENGINEER

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Tissue engineering (TE) and Nanotechnology are important researches fields to the future in the development of biological models useful for the understanding wound healing process and the extracellular matrix activity and others biological activities [1]. Nanotechnology have been showed many new interesting results in the multidisciplinary fields specially as a promising candidates for improving superior biocompatibility of many drugs, and development of materials with different optical, catalytic and magnetic properties compared to conventional materials (micron structured) [2]. In this context this work report the development of an *in vitro* three-dimensional human skin model denominated Dermal Equivalent (DE) with similar properties of human dermal skin layer for biological photoevaluation of the skin layers as the extracellular matrix after treatment with Low-Level Laser Therapy (LLLT) associated with a new nanostructured photosensitizer (PN) [2] (Fig. **1**).

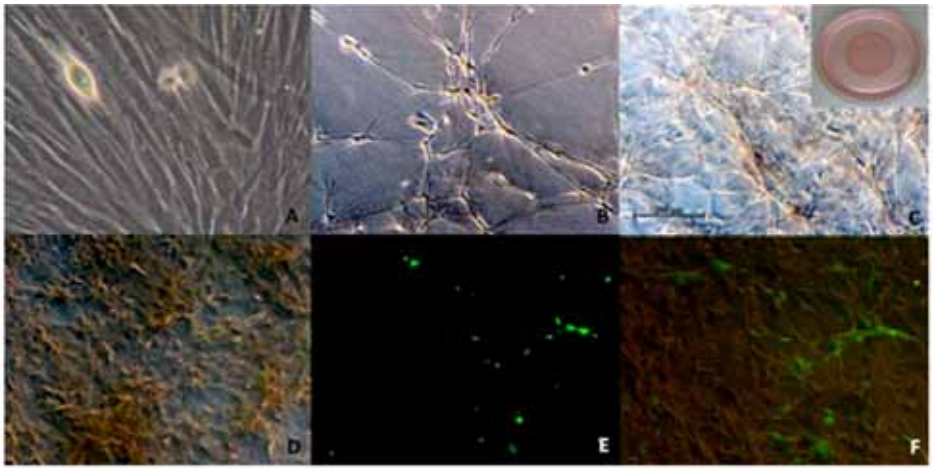

Fig. (1). Collagen matrix micrographs of DE and DE-BMMSC culture. Fibroblast monolayer culture (Fig. **1-A**); 3D-network collagen in 3 days (Fig. **1-B**); 3D-network collagen in 7 days and inset DE disc (Fig. **1-C**); dense 3D-network collagen covered with BMMSC (Fig. **1-D**); GFP-BMMSC fluorescence microscopy with excitation at 395 nm and emission at 509 nm (Fig. **1-E**) and GFP-BMMSC and 3D collagen overlapping.

The physical-chemistry characterization of nanomaterial developed showed a particle size of 239 nm (PDI = 0.22) and zeta potential of -46.2 mV, with all spectroscopic properties necessary for an effective PN. After LLLT stimulation the biological model of DE shows a differentiated kinetics of kinetic collagen matrix contraction of DE treated with PN at 10.0 μmol.L^{-1} and light activation at 40 mJ.cm^{-2} and 70 mJ.cm^{-2}. Besides was possible also to evaluate the Photodynamic effect on MMP-9 and MMP-2 (metalloproteinases enzymatic activity) secreted in DE supernatant medium after laser activation. Densitometric analysis of MMP-2 and MMP-9 bands on zymograms gel shows the increased of the enzymatic expression for low laser dose (at 40 mJ.cm^{-2}). These studies point out that this innovative therapeutic protocol based in the association of Photodynamic Process and Nanotechnology applicable with good results for skin wound healing extending the use of the Dermal Equivalents as biological models in the TE protocols.

Keywords: Tissue engineering (TE) and Nanotechnology.

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IL-54

Track: *Anti-Cancer Drug Discovery & Therapy*

EXPRESSION OF TRANSPORT PROTEINS FROM THE OATP FAMILY IN SOLID TUMORS: POTENTIAL TARGETS FOR TUMOR THERAPY

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Members of the human organic anion transporter (OATP) family are known to mediate the Na+-independent transmembrane transport of endogeneous and exogenous compounds into normal and cancerous cells. Through modifying the cellular accumulation of steroid conjugates, thyroid hormones, prostaglandins as well as peptide and anticancer drugs, they may influence cancer progression. From the eleven OATPs, which are grouped into sex families, some members show a quite restricted pattern in certain tissues and organs (e.g. well studied OATP1B1/OATP1B3 in liver, OATP4C1 in kidney, and OATP6A1 in testis), while others are more widely distributed. In cancer, the distribution pattern is no longer maintained. For example, the liver-specific OATP1B3 is down-regulated in hepatic tumors, but become upregulated in many cancerous tissues (colon, breast, prostate). Moreover, in a recent study, it was shown that OATP1B3 expression increases with a higher degree of differentiation in these cancers, whereas other OATPs, like the steroid-hormone transporting OATP2B1, are downregulated. Therefore, OATP1B3 may be considered as a diagnostic marker in these

tumors. For other OATPs, namely OATP2A1, OATP3A1, OATP4A1 and OATP5A1, which are only marginally expressed in normal liver, we could show a strong upregulation at the mRNA level by quantitative RT-PCR and at the protein levels by Western blotting. This was shown in hepatic tumors of different origin, e.g. hepatocellular carcinoma, cholangiocellular carcinoma, and liver metastases of colon tumors. Further studies using immunofluorescence microscopy and an automated image analysis system (TissueFAXS) revealed that these OATPs are present in tumor cells of epithelial origin. Additionally, they are also frequently present in other cell types, e.g. bile duct cells, endothelial cells and stromal cells. Also in specimens from other solid tumors, e.g. ovarian cancer, lung cancer etc., high expression levels of OATP3A1, OATP4A1 and OATP5A1 were observed, which reflect the degree of dedifferentiation of cells in these tissues. These data suggests that OATP3A1 and OATP4A1 might be considered as progression markers in certain cancers and their cellular distribution in tumorous tissues is assessed by a novel image analysis software (TissueQuest). High expression levels in samples from patients in hepatic and extrahepatic tumors were also observed for LST3-TM12, a novel OATP, which has not been studied at the protein level so far. LST-3TM12 is currently characterized for its functional properties using HEK cells transfected with this transporter. In summary, the altered expression of OATP like OATP3A1 and OATP4A1, which we show in solid tumors of different origin, suggests that these transport proteins could be important in cancer progression. They could modify the cellular accumulation of hormones, prostaglandins as well as anticancer drugs, thereby influencing the outcome of a certain tumor therapy.

IL-53

Track: *Innovative Drug Discovery and Nanotechnology*

MEMBRANE PROTEINS IN STABLE LIPID BILAYERS FOR FUNCTIONAL STUDIES AND APPLICATIONS

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Membrane proteins such as ion channels and transporters are major drug targets and their 3D-atomic structure resolution is currently in the focus of structural biology. To understand structure-function relationship, reliable assays are required. Most of current functional assays are based on cells or proteoliposomes. We are developing analytical systems consisting of self-assembled lipid bilayers beearing the membrane protein of interest separating two compartments. Since lipid bilayers are notoriously fragile and integration of functional membrane proteins is difficult, we evaluated in last years various supports of precisely defined pore diameters down to 200 nm in order to achieve stable free-standing lipid bilayers with integrated functional membrane proteins. Such chips of different pore arrays in silicon nitride membranes and also in polymer foils have been integrated in microfluidic systems. We developed suitable methods for lipid bilayer formation, integration of ion channels and electrochemical measurement of single protein channel activities. We now are entering in the phase of applications for relevant target proteins. Such novel biosensor systems are potentially useful for lead identification in drug discovery and furthermore to assess cell-uptake of drug compounds by measuring permeation across lipid bilayer membranes.

Keywords: Membrane proteins, function, biosensors, nanostructures, electrochemical detection.

IL-30

Track: *Recent Advances in Spectroscopy*

LABEL-FREE RAMAN MAPPING OF SURFACE DISTRIBUTION OF PROTEIN A AND IgG BIOMOLECULES AND SELECTED BIOMARKERS

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We have demonstrated a nanoengineered substrate composed of micropatterned silver nanoparticles to be used for the label-free mapping of adsorbed biomolecules. We utilized surface-enhanced

Raman scattering (SERS) phenomenon to monitor the known bioanalytes, protein A, human immunoglobulin G (IgG), and several selected biomarkers. The SERS substrate was composed of a poly(alylamine hydrochloride) (PAH)/poly(styrene sulfonate) (PSS) layer-by-layer (LbL) nanocoating micropatterned with silver nanoparticles confined to microscopic stripes. Selective adsorption of biomacromolecules is facilitated by the amine-terminated LbL nanocoating, which prevents the surface adsorption of positively-charged protein A across the surface except on the patterned regions containing negatively-charged silver nanoparticles. This label-free SERS approach provides accurate, selective, and fast detection of protein A and IgG solutions with a nanomolar concentration, down to below 1 nM for IgG in solution. This method could also be utilized for the facile detection of proteins under in-field conditions as well as in clinical, forensic, industrial, and environmental laboratories.

Keywords: Biomolecules, nanoparticles, nanoparticles.

IL-48

Track: *Anti-Cancer*

NEUROGENIC INFLAMMATION AND CARDIAC DYSFUNCTION DUE TO HYPOMAGNESEMIA DURING EGFR-TYROSINE KINASE INHIBITION

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In animal models of hypomagnesemia due to magnesium-deficient diets early indicators of oxidative and nitrosative stress result from neuronal release of neuropeptides [1]. With progressive hypomagnesemia significant cardiac inflammation and apoptosis occur; these changes can be partially reversed by the N-methyl D-aspartate (NMDA) receptor blockade that prevents loss of substance P. In addition, treatment with SP receptor blockers reduces oxidative/nitrosative stress. Using echocardiography to assess cardiac systolic and diastolic dysfunction during hypomagnesemia we found significantly reduced ventricular fractional shortening and enhanced diastolic dysfunction, which were partially restored by SP receptor blockade [2].

In our recent studies with normomagnesemic rats blockade by an EGFR tyrosine kinase inhibitor, tyrphostin AG1478, decreased magnesium levels for more than a month at which time significant systolic dysfunction became evident along with a trend towards impaired diastolic function. Concurrently, increased elevations of 8-isoprostane and enhanced superoxide generation by blood neutrophils indicated significant oxidative stress during treatment.

Cancer chemotherapy agents that block both EGF receptors as well as the EGFR tyrosine kinase have had increased clinical applications; antibodies to EGFR (e.g. cetuximab) have been reported to cause hypomagnesemia in many patients treated for colorectal cancer [3]. Our findings of hypomagnesemia and cardiac dysfunction due to EGFR tyrosine kinase inhibition may have clinical relevance, particularly in those patients who require combination therapy that includes other magnesium wasting drugs like cisplatin, which may result in even more severe hypomagnesemia and possible potentiation of cardiac side effects.

Supported by: NIH RO1-HL-62282

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IL-58

Track: *Cardiovascular Drug Discovery & Therapy*

IRON-OVERLOAD CARDIOMYOPATHY: LYSOSOMOTROPIC MECHANISMS OF PROTECTION BY BETA BLOCKERS

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Congestive heart failure is a primary cause of morbidity in transfused beta Thalassemic patients due to the accumulation of iron**,** which may increase to 28-fold above normal levels in cardiac and other tissues. In particular, clinical side effects of both parenteral and oral chelating agents may result in non compliance, indicating that potential adjunct therapies may be relevant for these patients. Since lysosomes are a cellular storage site for excess iron**,** they may play a critical role in cardiovascular injury due to their ability to release iron that promotes production of reactive oxygen species (ROS). Lipophilic beta-blockers like propranolol can concentrate 100-fold in lysosomal compartments, where the normally acidic "lysosol" can be alkalinized to prevent low pH-dependent release of redox-active iron; this lysosomotropic property is also protective against ROS injury. When we exposed iron-overloaded cultured endothelial cells to exogenous ROS, significant cytoprotection occurred after preincubation with d-propranolol. Using iron-overloaded rats**,** we documented significant systemic oxidative stress which was inhibited by treatment with sustained-release implanted pellets of d-propranolol. Using echocardiography**,** we also found significant protection against the iron-mediated loss of systolic (% FS and LVEF) contractility. When NADPH oxidase knockout mice (gp91phox KO) were exposed to iron-overload for several weeks, significant protection was observed against the loss of cardiac function that was exhibited by iron-overloaded wild-type controls; these results implicated the critical role of NADPH oxidase in the genesis of contractile dysfunction and supported a role for drug therapy to inhibit ROS-mediated injury. In conclusion, these studies suggest that d-propranolol, which can be administered in substantially larger doses than d,l-propranolol due to a lack of beta blocking effect, may be useful as a cardioprotective adjunct therapy in patients with iron overload who have difficulty adhering to treatment with standard chelation drugs.

IL-123

Track: *Drug Metabolism*

TRANSLATIONAL DRUG DISCOVERY RESEARCH: INTEGRATION OF MEDICINAL CHEMISTRY, COMPUTATIONAL MODELING, PHARMACOLOGY, ADME AND TOXICOLOGY

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Drug discovery and development is a complex but sequential process from discovery to preclinical development, followed with clinical drug development. It has been estimated that approximately 87% of the phase III failures are accounted for either due to lack of efficacy (66%) or due to safety issues (21%). Majority of these failures are for compounds targeted for novel mechanisms of actions with unmet medical need, in particular, oncology and neurodegenerative disorders. Some of the reasons for these failures can be attributed due to lack of appropriate preclinical animal models, biomarkers/surrogate markers and effective pharmacokinetic (PK)-pharmacodynamic (PD) evaluation during early drug discovery. Translational research which integrates computer aided drug design (CADD), PK, PD, drug metabolism (DM), drug transport (DT) utilization along with biomarkers and humanized animal models are instrumental in making informed decisions from early drug discovery through clinical development. The ability to correlate drug effect through modeling and simulations starts from early drug discovery and preclinical evaluation including use of novel biomarkers. Such models validate the PK and PD relationships and provide a basis for their applications and guide the Phase I - Phase III clinical trials more effectively minimizing the late stage failures. Thus, this talk provides brief overview on means and measures that can be adopted to integrate early drug discovery research along with safety and toxicological evaluation for meaningful transition to drug development.

IL-119

Track: *Structural Biology*

TOWARDS UNDERSTANDING THE STRUCTURAL BASIS OF ARP2/3-MEDIATED ACTIN-BRANCH FORMATION

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The Arp2/3 complex nucleates the polymerization of actin filaments from the side of existing filaments to form dendritic actin networks that power cellular and intracellular motility. Isolated Arp2/3 complex exists in an inactive form; its activation and subsequent actin nucleation require binding of nucleation promoting factors NPFs. The structural pathway underlying Arp2/3 activation remains elusive despite significant efforts to determine the crystal structures of the isolated complex in various nucleotide-bound forms and attempts to determine the topology of NFP interactions by methods such as chemical crosslinking and NMR. The crystal lattice appears to trap Arp2/3 complex in its inactive conformation, so far making the nucleation competent conformation unavailable to X ray crystallography.

By harnessing the power of high-resolution electron microscopy, we previously determined the structure of the complete branch junction, capturing the Arp2/3 complex in a fully activated state (Rouiller *et al*, JCB 2008). Here, we present the first high-resolution structural information of a much-sought-after state along the actin branch formation pathway obtained by high-resolution electron microscopy and image reconstruction. Namely, we determined the 3D structures of Arp2/3 complex bound to three of its most prominent NPFs: N-WASP with its activator Nck, Scar-VCA, and full-length cortactin. Distance constraints derived by FRET independently verified the NPF location within the complex (Xu *et al*, EMBO J 2011).

Our study directly compares the structures of Arp2/3 complex when bound to it's three most prominent NPFs and rigorously demonstrates that binding of all three NPFs induces distinct structural changes in the Arp2/3 complex. We show that NPF binding directly promotes a nucleation competent conformation with the actin-related proteins Arp2 and Arp3 in an actin filament-like dimer arrangement.

Keywords: Structural Basis, Arp2/3-mediated.

IL-182

Track: *Diabetes and Obesity Drug Discovery & Therapy*

MEASUREMENT OF THE DIABETES ASSOCIATED ANTIGEN GAD 65 IN HUMAN SERA

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Background: GAD 65 was generally considered to be strictly intracellular. This is a misconception in immunology because such a strictly intra-cytoplasmatic antigen would never have access to the immune system. With the ultrasensitive FCS (fluorescence correlation spectroscopy) technology, GAD 65 could be found to exist in peripheral blood of patients with diabetes mellitus type 1. It was measured with a sensitivity of 2650 ng/ml (1 ng/ml = 10^{-9} g/ml) in the sera of adult and paediatric patients. To understand the procedure and development of immunization and therefore the occurrence of GAD 65 antibodies (GADA) in the blood, it was proposed that in an initial step the immune system is primed by the accessibility to GAD 65. The assumption was that the enzyme has to be in the human serum and should be found in higher concentrations before the onset of the disease.

Method: The proof and quantitative evaluation of GAD 65 in the human blood was done with ELISA (enzyme-linked immunosorbent assay) where it was detected by interaction with corresponding antibodies, labelled with an enzyme. In the ELISA setup, the peripheral blood of persons representing a cross-section of 72 samples in a blood bank was used.

Result: From the measurements result that GAD 65 exists, in various amounts, in the sera of the blood-donors with an average concentration of 58.00 ng/ml. The correlation analysis of samples stored at -80°C and at room temperature

demonstrates the stability of GAD 65 at room temperature. The correlation coefficient between the GAD 65 concentrations, from samples stored at room temperature and from the same samples after one week shows that the molecule remains stable.

Conclusion: Our results encourage us to propose the antigen GAD 65, due to its frequency in human sera in different concentrations and its stability, as biomarker for the early diagnosis of diabetes mellitus type 1 and related inflammations.

IL-137

Track: *Inflammation & Immunology*

A HUMANIZED ANTI-IL-6 RECEPTOR ANTIBODY FOR THE TREATMENT OF CHRONIC INFLAMMATORY DISEASES

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Interleukin 6 (IL-6) is one of pro-inflammatory cytokine and is contributed in the pathological function of chronic inflammatory disease and autoimmune disease. Therefore, IL-6 blockade, a humanized anti-IL-6 receptor antibody, tocilizumab was established, which inhibited IL-6 activity both *in vitro* and *in vivo* before utilizing clinical therapy.

Since 1999, we performed clinical studies against C astleman's disease (CD), rheumatoid arthritis (RA) and systemic onset of juvenile idiopathic arthritis (sJIA). Almost 95% clinical efficacy was obtained by tocilizumab for the patients with CD. Against the patient with sJIA who could not controlled with any previous medicine and TNF-α blockades, tocilizumab therapy showed 90% efficacy assessed by JIA core set. In the case of RA, clinical studies of tocilizumab were performed with 8,000. In results, tocilizumab therapy for RA is tolerated and a most effective therapy in sign and symptom and joint event among the biological reagents assessed by ACR or DAS 28 criteria, and Sharp score. Moreover, tocilizumab therapy showed remarkable clinical efficacy without combination of methotrexate. Not only clinical efficacy, but also most of laboratory findings were shown the improving and normalization of such as CRP, SAA, Fib, Hb, CH50, and MMP-3.

Since IL-6 blockage is effective for CD, RA, and sJIA, other IL-6 contributed chronic inflammatory disease may be targeted diseases of tocilizumab, such as chronic angitis, autoimmune disease, autoinflammatory diseases, inflammatory bowel disease, amyloidosis, Bechget's disease and so on.

SESSION LECTURES

SL-121

Track: *Recent Advances in Patient Treatment and Care*

GOAL DIRECTED FLUID THERAPY REVISED: INDIRECT MONITORING OF INTERSTITIAL FLUID ACCUMULATION DURING MINI FLUID CHALLENGES WITH CRYSTALLOIDS

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Goal directed fluid therapy (GDT) implies administration of relatively small volume fluid challenges targeting maximization of target parameters mainly related to cardiovascular performance. Conventional GDT protocols will be shortly reviewed in the context of reported controversial impact on outcomes of treatment. There is a major concern that maximization of cardiovascular performance can be achieved in expense of deterioration in body hydration processes. The decrease of intravascular fluid retention and increase of fluid elimination was previously reported in consecutive fluid infusions. However, monitoring of interstitial fluid accumulation rate during fluid therapy is not available in everyday clinical practice. New method - minimal volume loading test (mVLT) - for the evaluation and optimization of fluid status will be shortly introduced (US patent 2010; US and International patent pending, 2011). It deploys deviations of capillary plasmadilution during mini fluid challenges with crystalloids. Calculations are based on changes of SpHb^{TM} non-invasively measured under the finger nail (Radical-7, Masimo, USA). Results of validation studies in healthy volunteers and orthopaedic patients will be discussed. Revised clinical applicability of SpHb will be described in the context of mVLT and recent reports about its relationship with arterial/venous Hb. Vision of the intelligent fluid infusion system will be discussed.

SL-116

Track: *Recent Advances in Patient Treatment and Care*

TOWARD HANDHELD DIAGNOSTICS OF CANCER BIOMARKERS IN BREATH: MICROFABRICATED GAS CHROMATOGRAPHY SYSTEMS

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Gas chromatography is a popular analytical tool employed to analyze volatile and semi-volatile organic compounds. It is employed in environmental, healthcare, food, and petroleum industries. Conventional GC systems are heavy, power-hungry, table-top instruments preventing their use for on-site analysis. Moreover, long analysis time is a major disadvantage of these systems which makes real-time analysis unattainable. Furthermore, there exists a risk of contamination, decomposition, degradation, and loss of sample during storage and transport from the collection site to the laboratory. This has initiated growing efforts to bring the lab to the sample instead of taking the sample to the lab through realizing micro GCs (mGC) as sophisticated handheld chemical analyzers.

The function of a μGC is to provide handheld capabilities for quantitative analysis of complex mixtures in clinical setting with short analysis time and small amounts of consumables. The μGC system consists of components which enable the capture, injection, separation, and detection of gaseous mixtures. This paper will present the recent development in various components of mGC systems. Microelectromechanical systems (MEMS) technology has allowed their miniaturization as well as the realization of new architectures for handheld and cost-effective cancer screening devices which are expected to facilitate early cancer detection in physicians'offices.

SL-156

Track: *Diabetes and Obesity Drug Discovery & Therapy*

ROLE OF GRAPE SEED EXTRACT AS POPULAR MEDICATION IN DIABETES MELLITUS

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The effects of an aqueous extract of grape seed that used as popular medication in many country of world was studied in rats with streptozotocin-induced diabetes. Rattus norvegicus rats weighing 200–250 g were used in this study. They were reared in the animal house of the College continue from 10/5/2011 - 11/6/2011. The animals were fed ad libitum with normal laboratory pellet diet and water. Oral administration of grape seed extract (2 mg/250 g rat body weight) for 30 days resulted in a significant reduce in glucose, GPT, GOT, ALP and ACP level. The extract also causes a significant increase in reduction glutathione in the liver and kidneys of rats with streptozotocin-induced diabetes. These results concluded the antioxidant property of grape seed extract

Keywords: grape seed, popular medication, diabetes mellitus.

SL-170

Track: *Pharmaceutical Research and Development (Other Areas of Pharmaceutical R&D)*

THE USE OF ANALYTICAL CHEMISTRY TECHNOLOGIES DURING THE SYNTHESIS OF ACTIVE PHARMACEUTICAL INGREDIENTS

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Analytical chemistry play a vital role in the identification and quantitation of impurities generated during the synthesis of Active Pharmaceutical Ingredients (APIs). Impurities can be classified into three major categories: Organic, inorganic and phase impurities. Analytical chemists utilize different classical methods (e.g. wet chemistry techniques) as well as instrumental techniques (e.g. chromatography and spectroscopy) during their investigation. In this presentation, we will focus on the evaluation of organic impurities. The toxicity of these impurities dictates what levels they need to be controlled at. For instance, potential genotoxic impurities would have to be controlled to parts-per- million (ppm) levels and thus extremely sensitive analytical methods need to be developed. High throughput methods are typically developed for applications where the turnaround time is critical. Examples of high throughput and real time analysis including fast LC, GC, SFC, and multi-parallel analysis will be demonstrated. Additionally, challenging achiral (structurally related), chiral (positional and stereo-isomers), and mass spectrometry method development will be discussed.

SL-115

Track: *Translational Medicine*

TARGETING TUMOR MICROENVIRONMENT WITH SILIBININ: PROMISE AND POTENTIAL FOR A TRANSLATIONAL CANCER CHEMOPREVENTIVE STRATEGY

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Tumor microenvironment refers to the dynamic cellular and extra-cellular components surrounding tumor cells at each stage of carcinogenesis. It is now considered an integral part of carcinogenesis that plays critical role in tumor growth, angiogenesis, epithelial to mesenchymal transition (EMT), invasion, migration and metastasis. Beside its vital role in carcinogenesis, tumor microenvironment is also a better drug target because of its relative genetic stability with lesser probability for drug-resistance development. Therefore, we believe that cancer chemopreventive strategies targeting both tumor and tumor microenvironment would be better and effective towards preventing, retarding or reversing the process of carcinogenesis. Our completed and ongoing studies clearly show that cancer chemopreventive agent 'silibinin' targets tumor growth, angiogenesis, EMT, invasion, migration and metastasis. In my presentation, I will elaborate these studies with major focus on the effect of silibinin on tumor microenvironment. Results from these studies are highly encouraging as silibinin targets tumor microenvironment effectively and more importantly, at much lower

concentrations compared to its inhibitory effect on cancer cells. Silibinin is already in clinical trials, and based upon our results we recommend that its chemopreventive effectiveness should be verified through its effect on biological end points in both tumor and tumor microenvironment.

SL-77

Track: *Drug Discovery in Preclinical Research*

IN-VIVO ANTILEUKEMIC ACTIVITY OF GONIOTHALAMIN

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Goniothalamin is a biologically active styrylpyrone derivative isolated from various *Goniothalamus* sp., belonging to the Annonacae family. The compound is very cytotoxic towards various leukemic cell lines and caused the cell to die through apoptosis cell death mode. Apoptosis cell death was found to occur via activation of caspase-3/-7. *In-vivo* antileukemic activity of the compound in myelomoncytic leukemia induced Balb/c mice was found as effective as vincristine. 100% survival was observed in the leukemic mice treated with goniothalamin and 20% of mortality rate was observed in the leukemic mice treated with vincritine. The significant decreased in the weights of liver and spleen of leukemic mice was also observed. The percentage of blasts cell in blood, bone marrow and spleen smears in leukemic mice treated with goniothalamin was compared to untreated group.

Keywords: Antileukemic, Goniothalamin.

SL-192

Track: *Drug Discovery in Preclinical Research*

IN-VITRO AND *IN-VIVO* ANTILEUKEMIC ACTIVTIES OF NEW CASTLE DIESEASE VIRUS STRAINS AF2240 AND V4-UPM

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In this study, two NDV isolates, AF 2240 and V4-UPM were evaluated for their anti-leukemic properties against WEHI-3B mouse myelomoncytic leukemia cells. The cytolytic dose - fifty percent for WEHI 3B treated with AF 2240 andV4-UPM strains for 72 hours *in-vitro* were 2 and 8 HAU, respectively. Apoptosis death mode was observed on WEHI-3B cells that treated with both virus strains. Low cytolytic activity was observed when the viruses treated against mouse fibroblasts 3T3 cell line, mouse lymphocytes and human peripheral monocytes. *In-vivo* anti-leukemic activity of both NDV strains was evaluated on WEHI 3B cells induced leukemic BALB/c mice. About 40% of BALB/c mice inoculated with WEHI 3B without treatment died in four weeks. In contrast, no mice died when there were treated with arabinocytocine or NDV. The total white blood cell and percentage of blasts cell in the blood, bone marrow and spleen smears were significantly low in mice treated with the ara-C or NDV as compared to the mice group without treatment. Significant increased in spleen and liver weight were observed in mice without treatment whereas spleen and liver weights were significantly reduced in the mice groups treated with arabinocytocine or NDV. From the results obtained, the mice groups treated with both NDV stains AF 2240 and V4-UPM showed similar antileukemic activity as Ara-C.

Keywords: Antileukemia, NDV, WEHI-3B.

SL-143

Track: *Pharmaceutical Research & Development*

INCORPORATION OF MICRONISED POORLY WATER-SOLUBLE ADDITIVE INTO INDOMETHACIN FORMULATED TABLETS. EFFECT OF CARRIER TYPE, ADDITVE AND DRUG CONCENTRATION ON DISSOLUTION: IS IT DUE TO MICRONISATION?

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Introduction: There are recent indications from the literature that the addition of micronised poorly water-soluble calcium phosphate (CP) as ternary additive to a poorly water-soluble drug, indomethacin and coarse carrier had enhanced the dissolution of the drug from interactive mixtures (1), (2). However, there is need to explore this enhancement in formulated tablets and to test whether this effect is due to the micronisation of the additive.

Objective: The objective of this work therefore was to study the hypothesis that the incorporation of micronised poorly water-soluble material CP into formulated tablets that contain indomethacin and a coarse carrier will enhance the dissolution of indomethacin and that enhancement is due to the de-agglomeration effect of the micronised ternary additive CP.

Materials and methods: Interactive mixtures for tablets were prepared by weighing the required amounts of indomethacin, 10% Explotab⟩ as disintegrant and 0.5% magnesium stearate as lubricant and different concentrations of micronised CP as agglomerate modifier, and placing them between two equal layers of carrier (lactose-povidone granules, Emcompress⟩, Emcocel⟩ or Emdex⟩). Tablets were manually made by the direct compression method using a 3 - F type Manesty tableting machine (Manesty Instruments, England). The particle size distributions for different materials were measured using a Malvern Mastersizer S (Malvern Instruments, Malvern, UK). The dissolution of the formulated tablets was measured using an automated DT-6 Erweka dissolution apparatus (Erweka, Germany) following the USP paddle method with water as the dissolution medium. Dissolution data were modelled using Sigma Plot software.

Results: The effect of different concentrations of CP (1%, 5% and 10%), different concentrations of indomethacin (10% and 5%) and different carriers (Emcompress⟩, Emcocel⟩ and Emdex⟩) on the dissolution of indomethacin in formulated tablets were studied. Results showed an increase in dissolution with the increase of CP concentration, an increase in dissolution with the decrease of indomethacin concentration and a dramatic increase in dissolution with the incorporation of CP in the tablets over different carriers. Modelling the dissolution data for the three effects revealed that the estimated initial concentrations of dispersed particles (Cd) and the dissolution rate constant (Kd) for the formulated tablets containing CP were significantly larger than those for formulated tablets without CP ($P < 0.035$). Use of un-micronised insoluble excipients was found to increase dissolution; however, this effect was likely related to redistribution of the indomethacin onto the un-micronised excipients which acted as secondary carrier and provided greater surface area for indomethacin dispersion.

Conclusion: The incorporation of CP in the formulated tablets containing micronised indomethacin with the coarse carrier was likely to produce indomethacin-CP agglomerates with lower strength leading to decreased agglomeration and improved dissolution rates.

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SL-171

Track: *Regenerative Medicine*

DEGENERATION/REGENERATION AS A MECHANISM CONTRIBUTING TO THE EFFECT OF MANUAL ACUPUNCTURE-INDUCED INJURY ON RAT SKELETAL MUSCLE

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This study aims to further improve our understanding of the underlying mechanism of local injury that occurs after manual acupuncture needle manipulation and that initiates the muscl e degeneration/regeneration process, which is essential for muscle maintenance and adaptation. Skeletal muscle is maintained by resident stem cells called muscle satellite cells. These cells are normally in quiescent state, but following muscle injury they re-enter the cell cycle and execute the myogenic program resulting in muscle fiber regeneration. Our previous work in young rats demonstrated that acupuncture technique induced injury that activated the resident satellite (stem) cells leading muscle regeneration. Skeletal muscle regeneration is an adaptive response to injury that requires a tightly orchestrated event between signaling pathways activated by growth factor and intrinsic regulatory program controlled by myogenic transcription factor. We identified several gene expressions uniquely important for muscle regeneration in response to acupuncture treatment at different time course using different biological techniques, including Immunocytochemistry, western blotting and Real Time PCR. This study uses a novel but non-invasive model of injury induced by manual acupuncture to further our current understanding of molecular mechanism of muscle stem cells. From a clinical perspective, this model of injury induced by manual acupuncture may be easily translatable into clinical tool as an alternative to physical exercise for patients challenge by bed rest or forced inactivity

Finally This Knowledge gained from this research could be useful for studies of the local effects various modality approach induced injury such as kaii a traditional Arab method of healing by cauterisation which may enhanced muscle stem cells.

SL-84

Track: *"Anti-Cancer Drug Discovery & Therapy"*

SAFFRON: A POTENTIAL TARGET FOR A NOVEL ANTI-CANCER DRUG AGAINST HEPATOCELLULAR CARCINOMA

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The purpose of this investigation was to investigate the chemopreventive action and the possible mechanisms of saffron against diethylnitrosamine (DEN)-induced liver cancer in rats. Administration of saffron at different doses per day was started two weeks prior to the DEN injection and was continued for 22 weeks. Biochemical analyses of antioxidants, ELISA, western blotting, FACS and immunohistochemical methods were employed in this study. Saffron significantly reduced the DEN-induced increase in the number and the incidence of hepatic dyschromatic nodules of DEN-treated rats. The results of immunohistochemical staining of rat liver showed that saffron inhibited the DEN-mediated elevations in numbers of cells positive for Ki-67, cyclooxygenase 2, inducible nitric oxide synthase, nuclear factor-kappa Bp-65 and the phosphorylated tumor necrosis factor receptor. Saffron also blocked the depletion in the number of cells positive for TUNEL and M30 CytoDeath in liver tissues of DEN-treated rats. *In vitro* experiments carried out using HepG2 cells also confirmed these findings and showed inhibition of NFkB activation, increased cleavage of caspase-3, as well as DNA damage and cell cycle arrest upon saffron treatment. The present study provides evidence that saffron exerts a significant chemopreventive effect against liver cancer through inhibition of cell proliferation and induction of apoptosis.

SL-14

IDENTIFICATION OF BRAIN MASS LESIONS BY HIGH MR IMAGING TECHNIQUES; MR-SPECTROSCOPY, DWI AND ADC MAPPING, BRAIN PERFUSION. PRELIMINARY RESULTS.

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Objective: In this study our aim was to determine the diagnostic performance of DWI, MRP and MRS compared with conventional MR imaging, histopathological results and clinical-radiological follow-up of patients for the differentiation of benign and malignant lesions of brain.

Materials and Methods: 30 patients with intracranial lesions who were prospectively evaluated with contrast material enhanced MR imaging, diffusion weighted imaging (DWI), multi-voxel MR spectroscopy (MRS) and perfusion MR (MRP) imaging were included in this study. The lesions were classified as benign and malignant according to the radiologic findings. All imaging datas were compared with the histopathologic results and clinical-radiological follow-up.

Results: The radiological parameters were evaluated statistically, sensitivity and spesificity were calculated in order to differentiate malignant and benign lesions; the highest values of sensitivity and spesificity were %87-81 for CBV and %88-87 for Cho/Cr ratio (TE: 26-44 ms). Additionally, for the differentiation of malignant and benign lesions; DWI predicted %77 sensitivity and %75 spesificity, MRP presented a %91 sensitivity and %88 spesifity, MRS yielded %77 sensitivity and %63 spesificity.. The combination of DWI plus MRP, DWI plus MRS or MRP plus MRS regarded %100 sensitivity.

Conclusion: Conventional MR imaging is inadequate for the differential diagnosis of intracranial lesions. For the detection of malignant lesions; combined use of DWI plus MRP, DWI plus MRS or MRP plus MRS can be used because of their high sensitivities.

Keywords: brain, tumor, MR, diffusion, perfusion, spectroscopy

SL-24

Track: *Hot Topics in Drug Target*

THE NEWEST HYPOTHESIS ABOUT VITILIGO MOST OF THE SUGGESTED PATHOGENESES OF VITILIGO CAN BE ATTRIBUTED TO LACK OF ONE FACTOR: ZINC-A2-GLYCOPROTEIN

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Zinc-α2-glycoprotein (ZAG) is a recently identified adipokine, assigned to the chromosome 7q22.1. It is a multidisciplinary protein, which is secreted in various body fluids. The ZAG plays roles in lipolysis, regulation of metabolism, cell proliferation and differentiation, regulation of melanin synthesis, cell adhesion, immunoregulation, etc.

Vitiligo is the most common depigmenting skin disorder, characterized by acquired, progressive and circumscribed amelanosis of the skin and hair. It commonly begins in childhood or young adulthood. The pathogenesis of this disorder is uncertain, but it appears to be dependent on the interaction of genetic, immunological and neurological factors.

For the first time, we pointed the probable association between ZAG and vitiligo. Herein, I have investigated this association in different views. By confirming this association, a surprising progression will occur in the treatment of this prevalent debilitating disease.

Keywords: Vitiligo, pathogenesis, zinc- α2-glycoprotein, tumor necrosis factor-α, melanocytorrhagy, cell adhesion, chromosome 7.

SL-150

Track: *Hot Topics in Natural Products*

COMPARISON OF THERAPEUTIC EFFICACY OF TOPICAL CORTICOSTEROID AND ORAL ZINC SULFATE-TOPICAL CORTICOSTEROID COMBINATION IN THE TREATMENT OF VITILIGO PATIENTS: A CLINICAL TRIAL

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Background: Vitiligo is the most prevalent pigmentary disorder which occurs worldwide, with an incidence rate between 0.1-4 percent. It is anticipated that the discovery of biological pathways of vitiligo pathogenesis will provide novel therapeutic and prophylactic targets for future approaches to the treatment and prevention of vitiligo. The purposes of this study were evaluating the efficacy of supplemental zinc on the treatment of vitiligo**.**

Methods: This randomized clinical trial was conducted for a period of one year. Thirty five patients among 86 participants were eligible to entrance to the study. The patients in two equal randomized groups took topical corticosteroid and combination of oral zinc sulfate-topical corticosteroid**.**

Results: The mean of responses in the corticosteroid group and the zinc sulfate-corticosteroid combination group were 21.43% and 24.7%, respectively**.**

Conclusion: Although, the response to corticosteroid plus zinc sulfate was more than corticosteroid, there was no statistically significant difference between them. It appeared that more robust long-term randomized controlled trials on more patients, maybe with higher doses of zinc sulfate, are needed to fully establish the efficacy of oral zinc in management of vitiligo**.**

Trial Registration: chiCTRTRC10000930

SL-66

Track: "Anti-Cancer Drug Discovery & Therapy

TOWARDS IMPROVING THERAPEUTIC RESPONSE IN PANCREATIC CANCER

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Pancreatic cancer (PC) is the fourth most common cause of cancer-related deaths. Due to the deadly nature of this malignancy, testing entirely new treatment options is difficult to pursue. Specificity proteins (Sp) transcription factors play critical role(s) in the growth and metastasis of cancer. Sp proteins also regulate the expression of Survivin, a member of Inhibitor of Apoptosis Protein family that is associated with resistance to chemo- and radiation therapies and impacts the prognosis. We hypothesize that targeting Sp proteins may provide a powerful strategy for enhancing the response of standard therapy. Research from our laboratory and others showed that tolfenamic acid (TA), a small molecule (NSAID), inhibits cancer cell growth through suppressing Sp proteins and survivin. We reported for the first time that TA augmented the response of PC cells and mouse tumors to radiation therapy by inducing radiosensitization. We also tested efficacy of TA in a combination therapy along with chemotherapeutic agents, 5FU or Gemzar. This combination therapy resulted in higher anti-proliferative response *in vitro* and dramatically decreased (>70%) tumor growth in mouse models. These findings are crucial in developing novel strategies by identifying genes/molecular pathways susceptible to be targeted for improving the therapeutic response in PC patients.

SL-175

Track: *Pharmaceutical Research & Development (Generic Pharmaceuticals: Challenges and Opportunities)*

SOLID-STATE CHEMISTRY AND NEW DRUG FORMS

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The contribution reviews the results of using the concepts and achievements of solid-state chemistry for design and preparation of new drug forms, with a special emphasis on obtaining new forms without modifying the molecular formula of the active pharmaceutical ingredient. Examples will cover the following topics: 1) control of polymorphism of pharmaceutical substances; 2) pharmacetical co-crystals; 3) obtaining pharmaceutical compounds as amorphous solids; 4) solubilization of drugs; 5) control of particle size and shape distribution for pharmaceutical solids; 6) "dry" synthesis of pharmaceutical compounds, and their composites with excipients; 7) "dry technologies" of extracting biologically active compounds from natural resources and/or preparing their composites with excipients.

SL-131

Track: *Stereoselective Synthesis of Bioactive Compounds*

OPTICALLY ACTIVE COMPOUNDS: SUPRAMOLECULAR ABSOLUTE CONFIGURATION DETERMINATION AND PROSPECTS IN STEREOSELECTIVE CATALYSIS

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Absolute configuration determination and synthesis of optically active compounds have attracted much attention owing to its direct relevance to many natural processes and important implications in various chiroptical devices, sensors, modern molecular and supramolecular technologies, and pharmaceuticals. Our supramolecular chirality sensors for determination of the absolute configuration are based on simple achiral ethane-bridged bis-porphyrin hosts (1). These bis-porphyrins are able to sense chirality of various enantiomeric guests via formation of the stable 1:2 supramolecular chiral complexes. The particular chirogenic mechanism of asymmetry transfer from chiral guests to achiral hosts upon the host-guest interactions includes formation of a unidirectional screw in the extended anti conformation of 1. These events generate a noticeable (moderate-to-strong) exciton couplet circular dichroism (CD) signal in the Soret band absorption region of 1. The induced CD sign unambiguously correlates with the induced helicity allowing straightforward determination of the absolute configuration of various chiral guests and thus making it possible to apply 1 as effective and universal chirality sensors for different types of optically active compounds. Various external and internal factors controlling the chirality induction processes have been comprehensively investigated by using 1 including a phase transition effect resulting in a chirality sensor in the solid state. Furthermore, to expand chiroptical applicability some chiral thin films have been obtained on the basis of these supramolecular systems.

However, for more practical application of the chiral technology and particularly for stereoselective synthesis, a stable and robust chiral material is highly desirable. To this end, chirally modified metal surfaces are considered to be one of the most suitable candidates for such materials. Preliminary results showed a great promise of this approach. In particular, a racemic mixture of histidine exhibited a nearly mirror image in the CD spectrum upon interaction with a nickel surface treated with enantiopure antipodal tartaric acids (TA). Furthermore, this material also opens up intriguing prospects for new asymmetric catalysts. For example, a chiral modification of Ni powder with TA resulted in the up to 86% enantiomeric excess of the hydrogenation reaction of methyl acetoacetate. Further details and developments towards chiral materials on the basis of these metal-containing systems will be discussed.

Keywords: Stereoselective Catalysis, Chiroptical devices, Supramolecular systems.

SL-193

Track: *Pharmaceutical Biotechnology*

NOVEL MASS SPECTROMETRY TECHNOLOGY DEVELOPMENT FOR BIOPARTICLE MEASUREMENT AND PROTEOMICS APPLICATIONS

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Recently, we have developed a few novel mass spectrometry technologies which can be used for proteomics studies and biomarker as well as drug discovery. These technologies include (1) Cell/microparticle mass spectrometry (2) virus/nanoparticle mass spectrometer and (3) Portable biological mass spectrometer.

Cell mass spectrometer extends the mass region to be covered from 1 megadalton to 1016 Da. It improves the mass range by 10 orders of magnitude. Virus/nanoparticle mass spectrometer can be used to measure the uptake of the number of nanoparticles and virions by a cell. The quantity of drug by nanoparticle can be quantitatively determined. The portable biological mass spectrometer combines several ionization techniques into one compact portable device. This inexpensive instrument will represent the new generation of mass spectrometry which can cover the measurement range from atom to cell. It can also be used for biomarker and drug discovery. In biomarker study, we have been able to look for lung cancer biomarker from exhaled air. Future perspective will be discussed.

Biography

Chung Hsuan (Winston) Chen got his B.S. degree from Chemistry Department, National Taiwan University in 1969. He got his Ph. D in Chemical Physics from University of Chicago in 1974, Then, he went to Oak Ridge National Laboratory to become a research staff. In 1989, he became Group Leader of Photophysics.During his tenure at Oak Ridge National Lab, his major effort was placed on development of ultra-sensitive detection technology. They include the first detection of single atom and isotope-selective atom counting. He is also one of the pioneers in developing mass spectrometry for DNA analysis and sequencing. His recent research has focused on novel mass spectrometry technology developments including cell mass spectrometer, accelerator mass spectrometer and portable biomolecular mass spectrometer. In 2007, he was appointed as Director of Genomics Research Center in Academia Sinica. He also has adjunct professor appointment at Chemistry Department, National Taiwan University. He has published more than ~230 papers in referred journals. He obtained 3 R&D-100 (100 top inventions in the year world wide) awards, He was elected as Fellow of American Physical Society in 1993, and AAAS in 2009 due to his contribution on ultrasensitive detection technology development. He was elected as Member of Academia Sinica in 2010.

SL-135

Track: *CNS Drug Discovery & Therapy*

FAST VERSUS SLOW STRATEGY OF SWITCHING PATIENTS WITH SCHIZOPHRENIA TO ARIPIPRAZOLE FROM OTHER ANTIPSYCHOTICS

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Aim: To compare strategies differing in the speed of switching schizophrenia patients to aripiprazole from other antipsychotic agents, with dual administration for 2 weeks and then tapering off the current antipsychotic in fast (within 1 week) versus slow (within 4 weeks) speeds.

Methods: This 8-week, open-label, randomized, parallel study was conducted among 79 patients with a primary DSM-IV diagnosis of schizophrenia were randomly assigned to either the fast- or slow-switching group. Efficacy measurements included Positive and Negative Symptom Scales and Clinical Global Impressions. Drug concentration and cytochrome P450 *CYP2D6* and *CYP3A4* genotypes were also measured.

Results: The fast-switching (n = 38) and slow-switching (n = 41) groups were comparable in the demographic features and previous medications. The fast-switching strategy, as compared with the slow-switching one, had equivalent efficacy in negative symptoms reduction, along with better improvement in total cholesterol and triglyceride levels, but without detectable worsening in positive symptoms or other side effects. The blood concentrations of aripiprazole of all

the patients were in therapeutic range at day 56, with *CYP2D6*10* polymorphisms associated with aripiprazole concentrations.

Conclusions: A fast-switching to aripiprazole from other antipsychotics can achieve improvement in both negative symptoms and metabolic profile and increase patients' therapeutic compliance.

Clinical Trials Registration: ClinicalTrials.gov identifier NCT00545467.

SL-26

Track: *Medical Imaging*

STUDY OF LUNG CANCER METASTASIS TO ADRENAL GL ANDS AND TO THE SPLEEN

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The authors analyzed 1492 patients with lung cancer. In 12 patients with pathological lesions in the spleen and in 58 in the adrenal glands, fine needle biopsy was performed. In 58 cases with biopsy-confirmed primary lung cancer metastasis to the adrenal glands and 11 to the spleen were detected. All the single metastasis deposits in the adrenal glands were located on the same side as the primary lung tumor. Metastasis in the spleen originated only from the left lung tumor.

In cases with disseminated cancer, 5 patients had confirmed metastases in both adrenal glands, 1 patient with the cancer located in the right lung had metastatic lesion in the left adrenal gland.

In these cases metastasis in other organs were also detected. The authors came to the conclusion that the direction of the metastatic dissemination depends on the way that the cancer spreads. The metastatic deposits, which occur by the spreading of cancer cells via the lymphatic, tend to locate on the same side according to the anatomy of the lymphatic system, whereas disseminated metastases seem to be hematogenous.

Keywords: Lung cancer, Metastasis, Pathological diagnosis, Ultrasound, Computer Tomography, Fine needle biopsy.

SL-36

Track: *Recent Advances in Spectroscopy*

MODELING OF MOLECULAR AND CHIRAL RECOGNITION BY CYCLODEXTRINS

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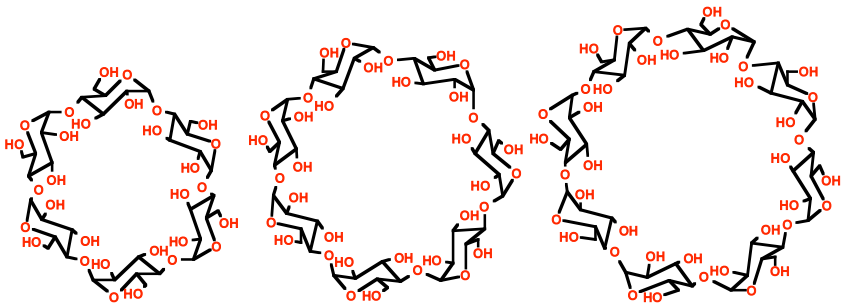
Cyclodextrin, CD **1** - **3**, complexes[1] have found numerous applications in pharmaceutical as drug carriers and for analytical purposes[2]. Therefore, modeling the complex formation[3] is not only of theoretical but also of practical importance. In particular, determination of relative stability of diastereomeric cyclodextrin complexes with enantiomers of drugs is of great value for pharmaceutical industry in view of their different biological activity and possible side effects of the second enantiomer of the drug. Accuracy and limitations of various methods used to model molecular and chiral recognition by cyclodextrins need to be assessed in view of complexity of these molecules and that of their complexes. The modeling of molecular and chiral recognition by CDs is often applied (36 papers published this year according to *Chem. Abstr.* in spite of warnings of the limitations of the calculations on CDs and their complexes[3]). The modeling is mostly based on the erroneous evaluation of the method given by Lipkowitz[4,5].



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SL-144

Track: *Women's Health Drug Discovery and Therapy*

POSSIBLE ANTI-UTERUS CANCER POTENTIAL OF POTENT HERB *ECLIPTA ALBA* AS EXHIBITED THROUGH ANTI-ESTROGENIC ACTIVITY

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In postmenopausal women, osteoporosis is one of the many complications. Estrogen supplementation through exogenous hormone supply helped to overcome the complications to some extent but was found to be associated with the development of uterus and breast cancer. Introduction of antiestrogen tamoxifene, has been a major success in treating estrogen (through Hormone Replacement Therapy) triggered cancers. Antiestrogens deprive the cells from estrogen by acting as estrogen receptor antagonists. The search of natural products for such compounds which may act as estrogen agonists or antagonists is interesting topic. *Eclipta alba* is a herb which is an important ingredient in many Chinese and herbal formulations which are in traditional use for the treatment of breast and uterus cancer, for the treatment of uterine bleeding, for regulating menstruation and several other complications related to female reproductive system. The literature survey encouraged us to test this plant for its estrogenic and antiestrogenic activities. Ishikawa assay is an enzyme based assay which is used to test the cytotoxic potential of samples alongwith estrogenic and antiestrogenic activity. This assay is preferred over all other available assays due to its rapidity, ability of cell line to mimic the human metabolism. Alkaline phosphatase (AP) activity in these cells is markedly increased when p-nitrophenylphosphate is converted to p-nitrophenol when estrogens bind to estrogen receptors (ERs) in the cell membrane and decreases in case of antiestrogens. Therefore estrogenic/antiestrogenic activity can be measured easily using this enzyme-substrate reaction in Ishikawa cell system. In the presented study, the crude extract of Eclipta alba (20µg/ml) exhibited significant antiestrogenic activity (53%). Among all the fractions of *Eclipta alba*, two fractions (F3&F4) showed antiestrogenic activity (94% & 96%) comparable to the antiestrogenic activity of positive control tamoxifen (96% inhibition at 5µM). The findings suggest that *Eclipta alba* has anti-uterus cancer potential which is mediated through the inhibition of alkaline phosphatase enzyme bringing about the anti-proliferation of endometrial carcinoma cells.

Acknowledgement: We highly appreciate Drug Discovery Centre at College of Pharmacy, University of Illinois at Chicago, USA for provision of facilities to perform Ishikawa assay. We also highly acknowledge Riphah International University and Higher Education Commission Pakistan (HEC) for providing funding for Participating in 4th International Conference on Drug Discovery and Therapy.

Keywords: Antiestrogenic activity, *Eclipta alba*, Ishikawa assay.

SL-211

EFFECTS OF REACTIVE OXYGEN SPECIES AND RADIATION ON BIOMOLECULES

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The impact of reactive oxygen species (ROS, highly reactive radicals such as •OH and O_2•-) and more stable species such as H_2O_2, e.g. produced by X-irradiation, and of UV radiation on many low-molecular and macromolecular compounds and fluids of biological interest has been investigated by various physicochemical techniques. In particular, spectroscopic studies of many proteins, polysaccharides and eye components, revealed, effectively and very fast, numerous changes of the local and global structure of the constituents under analysis, together with alterations of their functional ability.

By means of certain measures and a variety of additives (e.g. antioxidants), manifold modifications of the impact of ROS and of ionizing and nonionizing radiation can be achieved. Caused by differences in the primary reactions, biopolymers are protected effectively by typical •OH scavengers against ROS and X-irradiation, whereas compounds exhibiting significant absorption behavior in the UV range ("chemical filters"), and, in some cases, also the O_2•- scavenger superoxide dismutase (SOD), turn out to act as potent protectives against UV light. A few substances provide protection against both sorts of radiation and are even able to provide a chemical repair of already damaged particles.

Combining the above results regarding damaging events and protection and repair possibilities of biomolecules and the professional know-how of radiation and photo chemistry provides the scientific basis for understanding the occurrence of a variety of effects of ROS and radiation on biomolecules on the molecular level, together with possibilities to avoid or modify the detrimental effects. The application of (bio)chemically relevant types and concentrations of additives (e.g. (di)thiols, ascorbate, formate, NADH, SOD) allows the suppression of the noxious effects of radiation to a large extent. The results obtained are also of importance for understanding and avoiding pathological alterations of biomolecules and for developing new strategies for the protection and repair of biomolecules, e.g., in context with the bioprotection of eyes and skin and the aging of biomolecules or the application and development of nutritional supplements and drugs. Currently, the finding of precautions to prevent or alleviate the damages caused by the Fukushima fallout (in particular of the long-living radioactive isotope Cs-137, a gamma-ray emitter) would be of utmost importance.

Keywords: Reactive oxygen species, irradiation, screening techniques, damages of biomolecules, protection and repair strategies, antioxidants.

SL-187

Track: *Drug Discovery in Preclinical Research*

L-ARGININE AMELIORATES INTRALIPID-INDUCED DYSLIPIDEMIA AND HYPERGLYCEMIA IN EXPERIMENTAL ANIMALS

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Background: This study was undertaken to investigate the possible beneficial effects of exogenous L-arginine in ameliorating or regulating dyslipidemia and hyperglycemia induced in experimental animals.

Methods: Male Sprauge-Dawley rats were rendered dyslipidemic and hyperglycemic by intravenous injection of 20% intralipid (8.5 ml/kg b.w.) daily for three weeks. Plasma levels of glucose, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), very low-density lipoprotein cholesterol (VLDL-c) and free fatty acids (FFA) were measured in rats that were i.v. injected with 20% intralipid for 21 days in the presence and absence of exogenous L-arginine.

Results: After intralipid administration, plasma levels of glucose, TC, LDL-c, TG, VLDL-c and FFA were significantly increased, whereas HDL-c levels were significantly decreased. However, the intraperitoneal injection of L-arginine (500 mg/kg b.w.) for two weeks, either before or after intralipid administration maintained all parameters studies at or near control values.

Conclusion: These data provide evidence of the beneficial effects of exogenously administered L-arginine in normalizing experimental dyslipidemia and hyperglycemia in rats. These beneficial effects might be due to its direct NO-dependent pathway.

Keywords: Dyslipidemia, hyperglycemia, intralipid, L-arginine, nitric oxide.

SL-23

Track: *Hot Topics in Natural Product*

BENEFICIAL EFFECTS OF OMEGA-3 FATTY ACID DIETARY SUPPLEMENTATION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic lupus erythtematosus (SLE) is an autoimmune disease affecting many body tissues. Conventional treatment involves drug therapy with the risk of side effects. The use of dietary supplements gained a great interest in the last few years in order to minimize the adverse side effects of the drug therapy. The objective of this study was to investigate the possible beneficial effects of omega-3 fatty acid dietary supplement as an adjuvant therapy in combination with conventional drugs in patients with SLE.

Patients and Methods: Twenty-two SLE patients were divided into two groups. Group 1 (n=15) received the conventional drug therapy (prednisone + azathioprine) in combination with omega-3 fatty acid supplements for 8 weeks. Group 2 (n=7) received only (prednisone+azathioprine) for 8 weeks. Group 3 involved 10 healthy control subjects. Blood samples were collected from SLE patients at presentation and 4 and 8 week intervals after treatment and once from the control subjects to measure levels of ANA, anti-ds-DNA, TNF-α, IL-6, TBARS and lipid profiles as well as the activity of SOD and glutathione peroxidase in serum in addition to ESR.

Results: There was no significant difference between levels of ANA and anti-ds-DNA in group 1 compared to group 2. Levels of TNF-α and IL-6 in both SLE groups showed a significant increase compared to controls, and their levels were markedly decreased after 4 and 8 weeks in group 1 compared to group 2. TBARS levels increased significantly in both groups of SLE patients compared to controls, and decreased significantly in group 1 compared to group 2 after 8 weeks. Also, SOD and glutathione peroxidase activities were significantly decreased in both groups of SLE patients compared to controls, and increased significantly in group 1 compared to group 2 after 8 weeks. There were significant decrease in triglycerides and VLDL-cholesterol levels and a significant increase in HDL-cholesterol levels in group 1 compared to group 2 after 8 weeks.

Conclusions: This study suggests the beneficial effects of omega-3 fatty acid dietary supplements as an adjuvant therapy in combination with the conventional drug therapy in the control and management of SLE.

Keywords: SLE, Conventional drug therapy, Omega-3 fatty acids.

SL-83

Track: *Drug Discovery in Preclinical Research*

ON THE PROTECTIVE EFFECT OF OMEGA-3 AGAINST PROPIONIC ACID-INDUCED NEUROTOXICITY IN RAT PUPS

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Backgrounds: The investigation of the environmental contribution for developmental neurotoxicity is very important. Many environmental chemical exposures are now thought to contribute to the deve lopment of neurological disorders, especially in children. Results from animal studies may guide investigations of human populations toward identifying environmental contaminants and drugs that produce or protect from neurotoxicity and may help in the treatment of neurodevelopmental disorders.

Objective: To study the protective effects of omega-3 polyunsaturated fatty acid on brain intoxication induced by propionic acid (PPA) in rats.

Methods: 24 young male Western Albino rats were enrolled in the present study. They were grouped into three equal groups ; oral buffered PPA- treated group given a nuerotoxic dose of 250 mg/Kg body weight/day for 3 days ; omega-3 - protected group given a dose of 100mg/kg body weight/day omega-3 orally daily for 5 days followed by PPA for 3 days, and a third group as control given only phosphate buffered saline. Tumor necrosis factor-α , caspase-3, interlukin-6, gamma amino-buteric acid (GABA), serotonin, dopamine and phospholipids were then assayed in the rats brain's tissue of different groups.

Results: The obtained data showed that PPA caused multiple signs of brain toxicity as measured by depletion of gamaaminobyteric acid (GABA), serotonin (5HT) and dopamine (DA) as three important neurotransmitters that reflect brain function. A high significant increase of interlukin -6 (Il-6), tumor necrosis factor-α (TNF-α) as excellent markers of proinflammation and caspase-3 as a proapotic marker were remarkably elevated in the intoxicated group of rats. Moreover, brain phospholipid profile was impaired in PPA-treated young rats recording lower levels of phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylcholine (PC).

Conclusions: Omega-3 fatty acids showed a protective effects on PPA - induced changes in rats as there was a remarkable amelioration of most of the measured parameters (i.e. higher GABA, 5HT, DA, PE, PS and PC) and lower Il-6, TNF-α and caspase-3.

Keywords: Propionic acid, Cytokines, Caspase, Phospholipids, Neurotransmitters, Omega-3.

SL-93

Track: *Cardiovascular Drug Discovery & Therapy*

SIGNIFICANT DIFFERENCES BETWEEN LDL-CHOLESTEROL LEVELS OBTAINED BY FRIEDEWALD FORMULA AND A DIRECT METHOD

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Estimation of low density lipoprotein cholesterol (LDL-C) level in serum is considered t o be the basis of classification and management of hypercholesterolemia. In clinical laboratories, LDL-C cholesterol is usually estimated indirectly with the Friedewald's equation or more accurately with direct methods. The lack of agreement between the two methods has been reported in several clinical laboratories using different methods.

The present study is designed to compare LDL-cholesterol values obtained by Friedewald's formula and a direct method available in our laboratory (Dimension, RxL, SIEMENS). In the present study, we have found no significant differences between LDL-C obtained by Friedewald's formula (94.49 mg/dl ±28.81) and those determined by the direct method (93.98 mg/dl ±27.77) from samples with TG levels at ≤100 mg/dl ($p>0.4$) with correlation coefficient of 0.86. The LDL-C levels produced by Friedewald's formula were significantly lower than those obtained by the direct method when serum TG levels at 101-200 mg/dl ($p <0.001$) and 201-300 mg/dl ($p <0.01$) with correlation coefficient of 0.96 and 0.97 respectively. These differences are in agreement with those previously reported results in other laboratories. Therefore Friedewald's formula must be replace by the direct method for better classification and management of hypercholesterolemia.

SL-147

Track: *Inflammation & Immunology*

GENE POLYMORPHISMS OF TNF-α AND IL-10 RELATED TO RHEUMATIC HEART DISEASE

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Background: Rheumatic fever (RF) is inherited as a single recessive gene. Several genes are Likely to predispose an individual to develop rheumatic fever and rheumatic heart disease (RHD). Polymorphisms of TNF-α gene were associated with susceptibility to develop RF. T cells from all rheumatic fever patients produce significant amounts of TNF-α in response to steptococcal peptides with the highest production attained by the chronic rheumatic heart disease patients,and IL-10 expression was characterized in heart tissue of RHD patients by immuno-histochemistry.

Aim: To test the relation of RHD and gene polymorphisms of pro-inflammatory cytokines TNF-α gene at position -308 and anti -inflammatory IL-10 gene at position -1082.

Patients and methods: This study included 20 children with chronic rheumatic heart disease (group A) and 10 healthy children as a control group (group B). Patients group was classified into patients with single, and multiple valvular lesions, both of them were classified according to the severity by Echocardiography into : Group I : mild valvular lesion (n=7) Group II : moderate lesion (n=4) Group III : severe Lesion (n=9) Real time PCR was done for both TNF-α at-308 and IL-10 at position - 1082.

Results: All cases showed significant higher frequency of TNF-α homozygous genotype G/G compared to control group (P<0.05,OR,11). Cases with severe valvular lesions showed increased frequency of homozygous genotype G/G and increased frequency of IL-10 genotype G/A in cases compared to control group (p≤ 0.05,OR 13). There was no statistically significant difference in frequency of IL-10 genotypes among cases and control groups regarding to severity of valvular lesions. Composite genotypes (TNF-α G/G,IL-10 G/A) were higher in cases compared to control group (P≤ 0.01), while composite genotypes (TNF-α G/A,IL -10 G/G) were higher in control group compared to cases groups.

Conclusions: Susceptibility to RHD is associated with cytokine gene polymorphisms of TNF-α homozygous genotype G/G at-308 and IL-10 G/A at 1082. Composite genotypes (TNF-α G/G, IL-10 G/A) had high risk for RHD, while composite genotypes (TNF-α G/A, IL-10 G/G) may be protective genotypes.

SL-113

Track: *In-Silico Drug Design and In-Silico Screening*

A NOVEL *IN SILICO* PROTOCOL FOR THE DEVELOPMENT AND EVALUATION OF NEW HCV POLYMERASE INHIBITORS

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Infections caused by hepatitis C virus (HCV) are a significant world health problem for which novel therapies are in urgent demand. Nonstructural (NS5B) viral protein has emerged as an attractive target for HCV antivirals discovery. Toward this target several series of NS5B inhibitors that showed good activity have been reported. One of the important series is the benzimidazole class of inhibitors as represented by the JTK-109. Being potent, we focused our study on this class by generating an activity prediction computational model. Due to crystal cracking issues, benzimidazoles were not co-crystallized with HCV polymerase enzyme and therefore previous models were only ligand-based (e.g. CoMFA). Consequently, we provided for the first time a validated structural model of JTK-109-polymerase complex. Based on this generated complex, we constructed the first structure-based activity prediction model for the JTK-109 benzimidazole class. The model is a workflow of receptor-based pharmacophore followed by constrained docking. The pharmacophore was used as discriminative tool to reduce chemical space with superior ROC of AUC=0.958. In addition, it was used to qualitatively rank compounds according to their activity. Docking was used to provide a receptor-based QSAR model that can predict the activity of the compounds quantitatively based on its good correlation with the PLP1 scoring function (r^2=0.61). The workflow was applied to identify new analogues by screening a virtual focused library that was generated using the fragment hopping approach implemented in the ReCore engine and based on the validated complex. 52 hits were retrieved that have better physicochemical properties than JTK-109 and good predicted activities.

Keywords: Docking; Pharmacophore; JTK-109; HCV polymerase; ReCore.

SL-8

Track: *Medical Imaging*

3D IMAGERY OF LIVE CELLS LABELED WITH GOLD NANOPARTICLES USING DIGITAL HOLOGRAPHIC MICROSCOPY

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We have developed a novel heterodyne digital holographic microscope to detect and localize in 3D nanometric-sized gold particles used as biological markers in live cells environments. Holograms are obtained using the phaseshifting technique that is performed using two accurately synchronized Acousto-Optic modulators. We have also elaborated an ingenious numerical reconstruction method that offers striking advantages: reconstructed images do not suffer from longitudinal distortions, reconstruction parameters are obtained without an additional experimental calibration, aberrations compensation as well as lens curvature corrections are automatically obtained.

Using the developed optical apparatus and numerical processing procedure, we have been able to image and localize, for the first time in the context of holographic microscopy, 40 nm gold particles attached to the integrin surface receptors of live 3T3 fibroblasts with a localization precision of 5 nm laterally and 100 nm in depth, when 32 images averaging is performed. Additionally, 3D mapping of the entire scattered field was achieved where the 3D exploration was performed within a relatively big volume ~ 90 μm^3. We have been able to characterize the scattering regimes of the gold markers and cellular structures by analyzing the 3D shape of the corresponding scattering patterns that are easily accessible by digital holography.

SL-142

Track: *Inflammation & Immunology*

SST4 SOMATOSTATIN RECEPTOR AS A PROMISING DRUG TARGET

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It has been established that neuropeptide somatostatin exerts anti-inflammatory effects acting on somatostatin 4 (sst4) receptors. With the help of (sst4-/-) gene-deleted mice, we provide evidence that this receptor has a protective role in inflammation models, several symptoms are more severe in the sst4 knockouts than in their wild-type counterparts. Since somatostatin itself is not suitable for drug development because of its wide spectrum and short duration of action, receptor selective, orally active non-peptide analogues could be developed as new candidates of anti-inflammatory drugs. Sst4 receptors were also identified in the retinal ganglion cells, suggesting their functional importance. Carrageenin-induced paw edema, mechanical hyperalgesia and inflammatory pain in the adjuvant-evoked arthritis as well as oxazolone-induced dermatitis were much greater in sst4-/- mice. Orally active sst4 receptor agonists significantly inhibited oedema induced by mustard oil in rats and mice. They were inefficient in sst4 knockouts. Airway inflammation and bronchial hyperreactivity elicited by intranasal lipopolysaccharide were also markedly enhanced in sst4 knockout mice including increased edema, cellular infiltration, goblet cell hyperplasia, myeloperoxidase activity and inflammatory cytokine expression in the lung. It is concluded that somatostatin is released during inflammation and evokes counter-regulatory effects through sst4 receptors. Sst4 receptor agonists could be effective anti-inflammatory agents.

SL-134

Track: *Women's Health Drug Discovery and Therapy*

INCREASED TOCOLYTIC EFFECT OF NIFEDIPINE BY $ABCG_2$ EFFLUX PROTEIN INHIBITOR KO134 IN RAT *IN VIVO*

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$ABCG_2$ transporter has been shown to transport a wide range of substrates (e.g. Ca-channel blockers) and assumed to function as an important mechanism limiting cellular accumulation. The aims of this study were: (1) determination of the $ABCG_2$ expression during gestation in the rat uterus. (2) Investigation of the uterus relaxant effect of $ABCG_2$ substrate nifedipine in the presence of $ABCG_2$ inhibitor KO134.

The ABCG2 expression was measured by RT-PCR and western blot analysis. The uterus relaxant effect of nifedipine was measured by *in vivo* experiments. Low levels of ABCG2 expression were found at early stage of pregnancy but at 15th gestation day a sharp increase was detected and reached its maximum at day 18 and decreased from day 20 to post partum which was similar to non-pregnant uterus. The efficiency of the nifedipine was significantly increased by the parallel administration of KO134.

Due to the inhibition of efflux protein, the uterus relaxant effect of nifedipine was increased. The administration of efflux pump inhibitors with tocolytic agents may be of novel therapeutic relevance in the management of preterm labour.

This work was supported by TAMOP 4.2.1/B-09/1/KONV-2010-0005 grant.

SL-78

Track: *Anti-Cancer Drug Discovery & Therapy*

SEQUENCE-DEPENDENT SYNERGISM FROM COMBINATIONS OF CISPLATIN AND DESIGNED TRANSPLATINUMS IN HUMAN OVARIAN TUMOUR MODELS

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Introduction: *Trans*-planaramineplatinum(II) complexes with carboxylate-based leaving groups provide a novel class of platinums with an altered spectrum of activity. Being readily soluble in water their bio-availability and *in vivo* activity are expected to be high. Also, the compounds are stable towards hydrolysis. The reason behind an altered spectrum of activity lies in the fact that whereas cisplatin bind with DNA to form mainly intrastrand DNA adducts, *trans*-platinums are expected to form mainly interstrand adducts. As the repair enzymes responsible for cisplatin resistance may not recognize the interstrand adducts formed by the *trans*-platinums, combinations of cisplatin and *trans*-planaramineplatinum(II) complexes may exhibit sequence-dependent synergism. This study deals with new *trans*-platinums and their combinations with cisplatin in ovarian tumour models.

Methods: Carboxylate ligands are introduced in designed *trans*-planaramineplatinums by replacing the chloride leaving groups. MTT reduction assay is performed to determine the growth inhibition after exposure of human ovarian cancer cells to single drugs or drugs in combination. Combination index (CI) is calculated to give a quantitative measure of combined drug action. In addition, interaction of the compounds with pBR322 plasmid DNA has also been investigated.

Outcomes**:** As expected, *trans*-planaramineplatinum(II) complexes having carboxylate leaving groups are more soluble in water. Combination indices show that the binary combinations of cisplatin with the *trans*-platinums produce both sequence- and concentration-dependent synergism in all the three human ovarian cancer cell lines. The results from the interaction of pBR322 plasmid DNA indicate that the compounds have been able to cause conformational changes in the DNA and also DNA damage.

Conclusion and recommendations: This study is significant as it offers a new way of enhancing platinum action in human ovarian tumours. Increasing volume of sale and increasing use in the clinic indicate that cisplatin and its analogues will continue to be used in the clinic for a long time to come.

SL-151

Track: *Nutraceutical Drug Discovery & Therapy*

A PHASE I SAFETY AND PHARMACOKINETICS STUDY OF WITHAFERIN-A IN PATIENTS WITH ADVANCED STAGE HIGH GRADE OSTEOSARCOMA

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A Phase I Safety and Pharmacokinetics Study of Withaferin-A in Patients with Advanced Stage High Grade Osteosarcoma.

Introduction: Many plant derived principles have shown excellent anti-cancer activity in the past. Withaferin-A, an active principle obtained from a traditional Indian herb known as Ashwagandha or the Indian ginseng, has been shown to prevent and cure urethane induced lung tumors in mice, and also inhibit growth of transplanted sarcoma in mice. In this study we evaluated the safety and pharmacokinetics of withaferin-A in patients with advanced stage high-grade osteosarcoma.

Methods: A phase I dose escalation study was planned using the classical 3+3 design (C33D). Dose escalation cohorts comprised of 90, 135, 180 and 270 mg of withaferin-A administered in two or three divided doses per day. Three patients were enrolled on each cohort and the last patient was observed for at least 30 days for any dose limiting toxicity before progressing to higher cohort. Pharmacokinetic samples were collected on day 1 and plasma levels were measured using a validated high performance liquid chromatography (HPLC) technique having a limit of quantitation of 50ng/mL. Patients were followed-up at 3 monthly intervals. Safety evaluation including clinical examination, detailed history of adverse events, LFT, RFT and complete blood counts were performed at each visit. Withaferin-A was administered daily till progression. CTCAE version 3.0 was used for grading adverse events. The study was approved by the Ethics Committee of Tata Memorial Hospital and registered in clinicaltrials.gov (ClinicalTrials.gov identifier: NCT00689195).

Results: Thirteen patients were enrolled on the study. One patient in the 200mg cohort had to be replaced because he was lost to follow-up after the first dose. The formulation was generally well tolerated. No grade 3 adverse events were observed. Common side effects observed were elevation of liver enzymes (5/12) and skin rash (2/12). Others include fatigue, anemia, fever, edema and diarrhea (one each). None of the patients had detectable levels of withaferin-A in circulation.

Conclusion: The formulation was well tolerated. However, withaferin-A appears to have low oral bioavailability. Further studies with improved formulations are warranted.

Keywords: Withaferin-A, Ashwagandha, Osteosarcoma, Safety, Pharmacokinetics

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SL-203

Track: *Drug Delivery & Targeting*

CHITOSAN BASED HYBRIDS FOR DRUG DELIVERY

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Chitosan (CS) is a biocompatible, biodegradable, and non-toxic natural polymer and has applications in wound healing, tissue repair, antimicrobial resistance, cell adhesion, and food delivery. In this presentation, we report the facile synthesis of hierarchical mesoporous bio-polymer/silica composite materials with bimodal mesopores using a dual-template of the cationic N,N,N-trimethyl chitosan (TMCs) and the anionic sodium dodecyl sulfate (SDS) via one-step synthetic strategy. The mesoporous bio-polymer/silica composites encapsulate a large number of guest drug molecules, Ibuprofen (IBU) or 5-fluorouracil (5-FU), due to their high surface area and pore volume. In addition, the mesoporous chitosan-silica composites also had a long term biocompatibility for the target release of the drug molecules to the CEM cells and MCF cells etc. as well as a pH sensitive controlled release behavior of the drug molecules. We also present functionalized graphene oxides (GO) with chitosan (FGOCs). FGOCs were found to significantly improve the solubility of the GO in aqueous acidic media. Functionalization chemistry of GO would impart solubility and biocompatibility of FGOCs in biological environment. IBU and 5-FU were loaded on the FGOCs sheets with simple physisorption via π-stacking and inter-atomic interactions. Controlled release behavior of the two drugs was investigated.

SL-13

Track: *"Anti-Cancer Drug Discovery & Therapy"*

PEPTIDE APTAMERS, INHIBITORS OF HSP27: NEW THERAPEUTIC AGENTS

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Human Heat shock protein 27 (Hsp27) is an antiapoptotic protein characterized for its tumorigenic and metastatic properties, and now referenced as a major therapeutic target in many types of cancer. Hsp27 biochemical properties rely on a structural oligomeric and dynamic organization. Downregulation of its expression by antisense oligonucleotide or small interfering RNA has proven their efficiency to counteract the antiapoptotic and protective properties of Hsp27 leading to chemo- and radio-sensitization effects.

We investigated, *in vitro* and *in vivo*, whether functionally inhibition of Hsp27 using the peptide aptamer (PA) strategy, sensitizes the radioresistant SQ20B head and neck squamous carcinoma cells to gamma-irradiation. PAs expression perturbed the dimerization and oligomerization of Hsp27, and acted as negative regulators of the antiapoptotic and cytoprotective activities of this protein. We characterized two aptamers PA11 and PA50 that functionally inhibit Hsp27 and sensitize cancer cells to apoptosis *in vitro* and *in vivo* by disrupting the biochemical functions of the protein.

Constitutive expression of PA 11 and 50 in SQ20B cells (survival fraction at 2 Gy (SF2) = 0.75) increased the clonogenic cell death after irradiation. The survival fraction at 2 Gy, used as an index of radiosensitivity, shifted from 0.75 Gy in SQ20B-PA control cells to 0.40 and 0.51 Gy in SQ20B-PA11 and SQ20B-PA50 cell lines, respectively.

In vivo, nude mice bearing heterotopic SQ20B PA-expressing cells xenografts showed a strongly reduced tumor growth of 80 and 50% in SQ20B-PA11 and SQ20B-PA50 tumor xenografts, respectively, compared to SQ20B-PA control mice. The underlying mechanism of action of both PAs in reducing the tumor development involved a significant alteration of tumor cell proliferation by blocking the cell cycle.

These findings confirm that Hsp27 is a therapeutic target in cancer and suggest that PAs could provide a potential tool to develop strategies for the discovery of Hsp27 chemical inhibitors.

SL-105

Track: *Translational Medicine*

PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE PLAYS AN IMPORTANT ROLE IN A MOUSE MODEL OF TRIGEMINOVASCULAR ACTIVATION

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Pituitary adenylate cyclase-activating polypeptide (PACAP) and its specific PAC1 receptor are present in sensory neurons and vascular smooth muscle. Since PACAP infusion was shown to trigger migraine in humans, we aimed at investigating the pathophysiological changes in the nitroglycerol (NTG; 10 mg/kg i.p)-induced trigeminovascular activation model using PACAP gene-deleted (PACAP-/-) and wildtype (PACAP+/+) mice.

NTG-induced light aversive behavior determined in a light-dark box was significantly reduced in PACAP-/- mice both in the early (0-30 min) and late phases (90-120 min) due to direct vasodilation and trigeminal sensitization, respectively. Meningeal microcirculation measured by laser Doppler imaging increased by 30-35% in PACAP+/+, but not in PACAP-deficient mice. C-fos expression showing early neuronal activation significantly increased after NTG in the trigeminal ganglia and nucleus caudalis in PACAP+/+, but not in PACAP-/- animals. The intensity of PAC1 receptor immunostaining was similar in these regions of both groups. PACAP-38 (300 mg/kg, i.p.) produced similar photophobia and meningeal vasodilatation to NTG only in PACAP+/+ mice. Neuronal activation significantly increased in the trigeminal ganglia of both groups, but only in the nucleus caudalis of wildtypes.

These are the first animal experimental evidence for PACAP playing a pivotal role in migraine-related trigeminovascular activation, particularly in central sensitization.

SL-157

Track: *Pharmaceutical Research & Development*

ABKAMA A TRADITIONAL DOSAGE FORM AS A SOURCE FOR NOVEL DRUG DISCOVERY

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Over the past century drug research has contributed the most to the progress of medicine. The value of remedies used in traditional medicine as a source of drug discovery cannot be neglected. With the advancement of throughput screening methods it is now easier to select the potential remedies in the traditional medicine as sources of new drug discovery. The new techniques have enriched the standardization armamentarium so that the traditional dosage forms could be applied in today's system of medicine. The formulations could be used as an evidence base topic for medical researchers opening new windows to therapeutics. Going through world history of medicine undoubtedly one of the most influential systems of medicine is Islamic medicine. The remaining documents contain valuable dosage forms and formulations with precise methods of preparation. *Abkama* is a liquid dosage form mentioned as an anti infectious formulation in many traditional medical thirties. Preparation of this formula is considered in this paper according to five most famous traditional Persian medical lists of registry of different times. It seems that the method of preparation is similar to the conventional fermentation. Its listed therapeutic uses and the method of preparation from different sources make us believe this formulation is a valuable source for biotechnology research in the discovery of new antimicrobial agents.

Keywords: Abkama, traditional medicine, antimicrobial agents.

SL-196

Track: *Inflammation & Immunology*

PCR BASED ASSAY OF INTERLEUKIN-10 GENE POLYMORPHISM AND ITS BLOOD LEVEL AS BIOCHEMICAL MARKERS AMONG EGYPTIAN ATOPIC PATIENTS

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Background: Interleukin 10 (IL-10) is known to play various roles in immune regulating and anti inflammatory responses. Several polymorphic sites within the promoter region of the IL10 gene have been described and have been linked to the expression of allergies and asthma.

Objective: Evaluation the role of IL-10 polymorphism at position -592 C/A in allergic diseases as a biomarker molecule in the pathogenesis of atopy in Egyptian population.

Methods: The allele frequency for one single base pair substitution at position -592C/A in the IL-10 gene promoter was determined in 100 volunteers; of these, 25 had atopic dermatitis, 25 had allergic rhinitis, 25 had atopic asthma, and 25 were normal controls using PCR and restriction fragment length polymorphism (RFLP). Serum IL-10 level, total IgE and allergen specific IgE were measured in every individual.

Results: IL-10 allele frequencies at position -592 C/A were different between control and all the atopic groups studied (asthma, dermatitis, and rhinitis) ($p < 0.001$, < 0.001, and < 0.001 respectively). However, the IL-10 values were not different among the control and all the studied groups ($F = 2.522$, $P = 0.062$).

Conclusion: IL-10 SNP at -592C/A is a candidate genetic marker to screen for the atopic group studied. However, the IL-10 level in the blood can't be used as an indicator for any group of atopy because its level in the blood is not affected only by its gene promoter polymorphisms which have been found to be associated with the IL-10 production. No significant correlation was found between IL-10 level in blood and IgE in any of the atopy groups studied; although IgE

was found to be phenotypic marker for all the atopy studied groups. This can be attributed to several factors affecting the IL-10 level in blood.

SL-155

Track: *Hot Topics in Natural Products*

IN VITRO ANTHELMINTIC ACTIVITY OF DIFFERENT FRACTIONS OF *AZADIRACHTA INDICA* A. JUSS SEEDS

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Control of gastrointestinal nematodes (GINs) in animals is crucial for their optimum productivity. Different anthelmintics are used for treating GINs. Development of resistance in helminths against the commonly used anthelmintics has, however, posed a challenge for the animal health workers throughout the world. Medicinal plants, therefore, have been focused as potential candidates for their use as anthelmintics. *Azadirachta indica* is amongst the long inventory of plants used as botanical anthelmintics in the folk veterinary medicine. This investigation was aimed at determination of anthelmintic activity of different fractions prepared from *A. indica* seeds crude aqueous methanol extracts. For this purpose, eggs and adult *Haemonchus contortus* were exposed to aqueous, ethyle acetate, chloroform and petroleum spirit fractions of *A. indica* seeds employing standard egg hatch assay and adult motility test at graded concentrations. All the fractions of *A. indica* seeds exhibited dose and/or time dependent (R^2= 0.9379-0.9883) ovicidal and wormicidal effects against *H. contortus*. The best ovicidal activity was demonstrated by the ethyle acetate fraction with LC_{50}= 21.32 µg/ml; whereas, it was 6-14 times lower for the other fractions. Likewise, ethyle acetate fraction @ 50 mg mL^{-1} exhibited the best wormicidal effects by killing 83% adult *H. contortus* one hour post-exposure.

SL-200

PLASMINOGEN ACTIVATOR INHIBITOR WITH VERY LONG HALF-LIFE (VLHL PAI-1) IN CANCER, PAI-1 DEFICIENCY AND DIABETES THERAPY

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Imbalance in proteolysis is in the foundation of many diseases. Upregulated urokinase (uPA) activity is responsible for angiogenesis and cancer metastasis and is a prognostic marker of many cancers. In plasminogen activator inhibitor (PAI-1) deficiency tissue plasminogen activator (tPA) unopposed by PAI-1 activity leads to frequent bleeding episodes. Overexpression of PAI-1 in diabetes leads to diabetic nephropathy in the late stages of this disease. Development of selective regulators of proteolysis would produce novel therapeutics. One of the possibilities would be to use PAI-1 itself. However, PAI-1 is not stable and converts itself into the latent form with a half-life of 2h. Retraction of the active center loop significantly rearranges protein and inactivates it. We have produced PAI-1 mutants by replacing chosen amino acids with cysteine to create disulfide bridges, which could make this insertion more difficult. One of such mutants, VLHLPAI-1 has extended half-life activity over 700h. PAI-1 with extended half-life reduces angiogenesis in animals and human models. Also, application of PAI-1 reduces significantly tumor size in mouse. Systemic or topical application of PAI-1 reduces bleeding. Other mutant of VLHL clearance of wild type PAI-1 from circulation PAI-1 (Arg360Ala) would increase and can slow down progression of diabetic nephropathy. Therefore, VLHLPAI-1 could be used as a therapeutic for diverse clinical applications.

Keywords: Proteolysis, Upregulated urokinase, cancer metastasis, plasminogen activator, disulfide bridges.

SL-122

Track: *Combinatorial Chemistry*

THE SYNTHESIS OF SOME TRICYCLIC BENZODIAZEPINONES

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Tricyclic benzodiazepine derivatives bearing a heterocyclic ring joined between positions 4 and 5 of the benzodiazepine moiety are described. The heterocyclic ring will contain the nitrogen atom appearing at position 4 of the benzodiazepine ring as well as the hetero atom, which may be either oxygen or nitrogen, attached to the carbon atom at the 5-position of the benzodiazepine ring are useful as sedative, muscle relaxant and anti-convulsant agents. In this research, a new method was offered for synthesis of 10-Nitro-11b-(2-fluorophenyl)-2,3,5,11b-tetrahydrooxazolo[3,2-d][1,4] benzodiazepine-6-(7H)-one and 10-Nitro-11b-(2-fluorophenyl)-2,3,5,6,7,11b-hexahydro-1H-imidazo[1,2-d][1,4] benzodiazepine-6-(7H)-one from 2-bromoacetamido-5-nitro-2'-fluorobenzophenone by Ethanolamine and Ethylenediamine. The starting material of this synthesis i.e 2-bromoacetamido- 5-nitro-2'-fluorobenzophenone was prepared from reaction of (2-amino-5-nitrophenyl)(2-fluorophenyl)methanone and 2-bromoacetyl bromide.

Keywords: Tricyclic benzodiazepine, 1,4-benzodiazepinones, Sedative – hypnotic, Ethylenediamine, ehtanolamine.

SL-136

Track: *Stereoselective Synthesis of Bioactive Compounds*

SYNTHESIS OF ENANTIOMERICALLY PURE SPIROOXINDOLES USING A THREE COMPONENT REACTION VIA 1:3 DIPOLAR CYCLOADDITION REACTION

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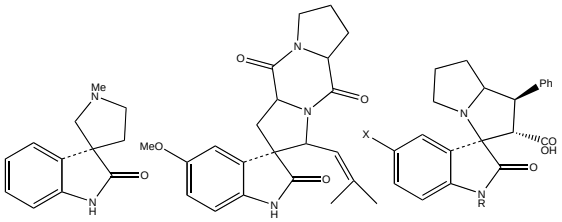
Oxindole derivatives are important targets in medicinal chemistry. A novel synthesis of spirooxindole derivatives has been developed. Specifically we were interested to devise a simple and enantioselective synthesis of compounds represented by general structure **3**. 1,3 dipolar cycloaddition of the nonthermally generated azomethine ylide to a α, β unsaturated compound with chiral auxilliary of menthol and oxazoline. Structures represented by **3** have many desirable characteristics of drugs, i.e. molecular weights < 500, presence of hydrogen bonding acceptor and donor, aromatic rings which could be suitably substituted. A basic nitrogen which as a salt will impart solubility and overall condensed ring system which impart geometry that might be significant in drug discovery.



(1) horsfiline (2) spirotryprostatin (3)

Keywords: Spirooxindole, 1, 3 dipolar cycloaddition, enantioselective synthesis, chiral auxilliary.

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SL-148

ANIMAL FECAL ACTINOMYCETES, A NEW SOURCE OF DRUG DISCOVERY

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Actinomycetes (Actinobacteria) have been paid a great attention owing to their production of various natural drugs and other bioactive metabolites including antibiotics, enzyme inhibitors and enzymes. Actinomycete is still an important source developing new natural drug. So Baltz showed a proposition of "Renaissance in antibacterial discovery from actinomycetes". However, the development of new drug from actinomycetes in common habitats is more and more difficult. In order to overcome these challenges, some new concept based on the genome was described, that is "new habitats, new methods, new species, new gene, new products and new use". In other words, novel species should contain new gene cluster synthesizing new secondary metabolites, so far as getting new species is an important premise for obtaining new compounds. Many companies and laboratories focus on new actinomycete sources from new habitats, oceans, extreme environment and plants for development of new drug. In our view, making the uncultured to pure cultured microorganisms is one new hope for getting new drug leader compounds.

Actinomycete, as a pathogen of human and animal, had been studied before. But the research work on it, as a source for discovery of novel leader of drugs, is very few. In order to get much more unknown actinomycetes for discovering new bioactive metabolites, the actinomycetes of 26 species of animal feces were isolated and identified. Anti-microbial activities, enzyme activity, and synthesis genes of five antibiotics of some selected strains were detected. Bioactive compounds of some trains were isolated and characterized. Some results are reported here.

In the course of co-evolution of microorganisms and their hosts, the former play an important role in digestion and absorption of food, immunity, resistance to pathogens, and maintaining health of host. In order to provide new source for discovering new leader compounds of drugs, the diversity and some bioactivities of cultured actinobacteria in animal feces have been studied. 26 animal fecal samples were collected from Yunnan Wild Animal Park. The purified cultures of actinobacteria were isolated from these samples by using 5 media. The 16S rRNA gene sequences of 344 selected strains were determined, the phylogenetic analysis was carried out, and activities of 19 enzymes, degradation of cellulose and feather, and anti-microbial activities were determined. Compounds having bioactivities were isolated.

33 genera of actinobacteria from 26 animal feces were identified. They were *Agrococcus, Arthrobacter, Cellulosimicrobium, Citricoccus, Corynebacterium, Curtobacterium, Dietzia, Enteractinococcus, Gordonia, Isoptericola, Janibacter, Jiangella, Kocuria, Labedella, Leucobacter, Microbacterium, Micrococcus, Micromonospora, Mycobacterium, Nocardia, Nocardiopsis, Oerskovia, Promicromonospora, Pseudonocardia, Rhodococcus, Saccharomonospora, Salinibacterium, Sanguibacter, Streptomyces, Tsukamurella, Verrucosispora, Williamsia and Yaniella*. The 33 genera of actinobacteria distributed in 7 Suborder, *Micrococcineae* (17 genera), *Corynebacterineae* (9 genera). *Pseudonocardineae* (2 genera), *Streptomycineae* (1 genus), *Streptosporangineae* (1 genus), *Micromonosporineae* (2 genera), and *Jiangellineae* (1 genus). No Suborder *Actinomycineae*, *Propionibacterineae*, *Frankineae* and *Glycomycineae* were isolated. Members of Streptomycetes were still preponderant in most animal feces, and cfu (colony-forming units) /g dried sample was 27 to 1767. Members of *Rhodococcus* were isolated and identified from 18 animal feces, and was one of the widest distribution in these animal feces. 22%, 13%, 6%, 20% and 16% of 384 tested strains had inhibition against *Bacillus subtilis*, *Staphylococcus lentus*, *Mycobacterium tuberculosis*, *Candida albicans* and *Aspergillus niger* respectively. Most strains had activities of various enzymes, including Esterase lipase (C8), Leucine, arylamidase, acid phosphatase, α-glucosidase, β-glucosidase and Naphthol-AS-BI-phosphohydrolase, and degradation of feather and cellulose. Fecal actinobacteria had also widely anti-tumor activity. Some of them had high activities, and the IC50 of crude extracts of some strains were below 4 μg/ml. Some fecal actinobacteria contained respectively genes of biosynthesis enzyme for PKS I, PKS II, NRPS and CPY.

More than 50 of known and new secondary metabolites with bioactivities, abkhazomycin, alazopeptin, apigenin, candicidin, desertomycin, desferrioxamine E, enopetin A/B, emodin, favofungin, geldanamycin, hopanoid, kasugamycin, kidamycins, longestin, rutamycin and polyene macrolides etc. were isolated and characterized from fecal actinomycete strains by cooperative partners. Structures and activities of these compounds are very diverse and complex. Sannastatin, a novel toxic macrolactam polyketide glycoside was produced by strains YIM 100282, an un-identified *Streptomyces* sp. Structures of other possible new compounds have been elucidated.

Therefore we believe that animal fecal actinobacteria, like those from soil, ocean, extreme environment and plant, are an important source for developing novel antibiotics, anti-tumor agents, enzyme inhibitors, enzymes and other industry products. We show further a hypothesis that bioactive metabolites produced by fecal actinobacteria and other microorganisms, except pathogen, should be no toxic or lower toxic at least to their hosts and human. Comparing with the metabolites from other sources, maybe this is a very important excellence.

Methods of obtaining un-known or novel actinobacteria from animal feces are described as following:

Existence of Gram negative bacteria in a large number in animal feces is a main problem for isolation of fecal actinobacteria. In order to eliminate the trouble of Gram negative bacteria, and obtain much more un-known actinobacteria for discovering novel leader compounds, sampling and isolation methods are key points.

Based on various tests in our laboratory, first, fresh feces samples had to been dried at 25-28 ºC for 7 to 10 days; second, pre-treatment of dried samples at 80ºC for 60 min has to be carried out before isolation; third, potassium bichromate 50mg, nystatin 50mg, nalidixic acid 20mg and 5mg penicillin for 1000ml medium, as inhibitors, have to be added in the isolation medium for inhibiting fungi and Gram negative bacteria; fourth, The dilution of samples should be 10-5, 10-6, and 10-7; fifth, YIM 212, YIM 171 and HV medium were better for isolation of actinobacteria from animal feces.

Keywords: Animal feces, Actinomycetes, Diversity, Bioactivity, Resource, drug discovery.

SL-146

Track: *Nutraceutical Drug Discovery & Therapy*

THE DISCOVERY OF THE EFFICACY OF LEPIDIUM SATIVUM SEEDS ON FRACTURE HEALING

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The journey to explore the impact of cereal plant "*Lepidium sativum* Seeds" began with the author for more than fifty years in *Mecca* when he was a child, grazing *Sheeps* for his family which was the custom of people in those days. Some of those sheeps sustained fractures in their limbs and he had to tr eat them personally by strapping the broken parts with 2 pieces of wood and a mixture of egg and starch included between the woods. In addition, those sheeps were fed with seeds of *Lepidium sativum* known locally as *THUFFAH* or else *Garden Cress*. Rapid healing of fractures was noticed by the author since then and continued for years. In the late seventies of the previous century, the author was capable of noticing the effects of *L. sativum* seeds on human beings having fractures and taking seeds with accelerated healing in those patients having acute, delayed union and non-union, but without obvious scientific data in literature to prove /or disprove this important observation. This encouraged the author to conduct clinical trials to some patients who suffered from acute injuries and non-union after surgical femoral correction for severe valgus deformity, and another patient who had non-union in fractures both radius and ulna with multiple surgeries and bone grafting ended by failure. Those 2 cases who refused further surgical interventions, accepted a clinical trial of ingestion of *L. sativum* seeds in a controlled adult daily dose for a specific time. The results were clinically significant with complete union in 4-6 weeks' time. Those personal author's results and surveillance with the clear reputation of the effects of *L. sativum* within the community encouraged him to document these very important significant clinical effects of the seeds. Not until the beginning of this century, that the author thought of a reliable method to carry out the experiment in laboratory on New Zealand rabbits to induce open fractures and internal fixation in femur conducted personally by him. This work was published internationally with great effects and impacts in the medical and paramedical fields [1].

Further contribution of this study to the medical literature was done in chapter "61" [2]. The conclusion of this work is that an author's surveillance of more than 50 years, with continuous testing and experiments, succeeded to prove scientifically that the effect of *Lepidium sativum* seeds, known as well, Thuffah or Garden Cress, is an important factor of a natural product with significant effects to accelerate healing of fractures in medical practice. It is an honour that this study stood proudly for the last 5 years since its date of publica on "2007" and guided lot of medical and nonmedical institutes globally to discover this fact and apply their own researches for similar and different effects and applications of the plant and seeds in their own fields. The discovery of this drug in this century has a great influence, not only, in Orthopaedics and Trauma but in many other diseases and human health.

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SL-174

Track: *Regenerative Medicine*

A VERY RARE COMBINATION OF NAIL – PATELLA SYNDROME (NPS) AND DOWN'S SYNDROME (DS): THE FIRST DESCRIBED STUDY

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The association between two rare genetic disorders such as Nail-patella Syndrome and Down's syndrome has not been reported in medical literature before. In this study, a five year old male patient presented to the Orthopaedic Clinic with knee complaint and abnormal gait. Clinically, both NPSand DSwere discovered from the phenotypes and radiologically,havinga positive family existence in one sister in NPS. The knee problem was due to absence of both patellae with marked laxity of ligaments. Follow-up and possible surgical interventions in future are the milestones for him.

NPS is a pleiotropic disorder exhibiting autosomal dominant inheritance as a result of mutations in the LIM-homeodomain gene, LMX1B, mapped on the long arm of Chromosome 9 with a high degree of variability in phenotypes. NPS is characterized, clinically and radiologically, by absence or hypoplasia of nails and patella, posterior iliac horns in more than 80% of cases, congenital nephropathy, cervical ribs, and eye problems. It is also known as hereditary onycho-osteo-dysplasia (HOOD), with thumb and index fingers mostly affected. Other abnormal features have been reported, such as antecubital pterygium, clavicular horn, foot abnormalities, spondylolisthesis, skull defect, absence of fibula, shoulder and first rib dysplasia, nephropathies and abnormal structures firstly described within the affected knees [1].

Whereas, Down's syndrome is aneuploidy genetic disorder due extra copy (complete or partial) of Chromosome 21 causing Trisomy 21.

Both conditions have minor similarities being genetically based disorders with positive family history, and equal in age, racial and gender factors BUT they differ significantly in the mechanism of genesis, karyotyping, phenotyping, rate of incidence (being rarer in NPS), and radiological features. Their treatment is multi-disciplinary and the Orthopaedic role, especially surgery, is important for the musculoskeletal disabilities [1].

In conclusion: The discovery in this study of these two rare genetic disorders occurring simultaneously in one patient is the first reported case in literature. Thisindicates the importance of chromosomal study in revealing the mechanism of occurrence, the association of disorders between Chromosome 9 and 21 and possible mode of therapy for them.

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SL-81

Track: *CNS Drug Discovery & Therapy*

EVALUATION OF ANTIDEPRESSANT EFFECTS OF CELECOXIB (COX 2 INHIBITOR) AND ITS COMBINATION WITH DULOXETINE (SNRI) IN STRESSED MICE

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Rationale: In recent years, a potential link between inflammation and depression has been shown and the role of pro-inflammatory cytokines in the pathophysiology of major depressive disorders has been observed. Celecoxib (selective COX 2 inhibitor) has been reported to inhibit the production of PGE-2 and proinflammatory cytokines and also increase tryptophan levels and serotonin availability in depressed patients. On the other hand, duloxetine (potent SNRI) has been shown to be efficacious in inflammatory and acute pain models in rodents and synergistic interaction with NSAIDs. However, the interactions of duloxetine with celecoxib are currently unknown.

Objective: We evaluated the antidepressant effect of celecoxib (15, 30 mg/kg/day for 15 days, ip) alone and in combination with duloxetine (5, 10 mg/kg/day for 15 days, ip) and also the biochemical parameters in stressed mice.

Results: Pretreatment of celecoxib (15, 30 mg/kg) for 15 days to forced swim-induced stressed mice produced significant antidepressant effect which has been evidenced by decreased in immobility time in tail suspension test (TST). Celecoxib (30 mg/kg) also showed significant increase in locomotor activity and protective effect on biochemical parameters of oxidative stress by reversing stress-induced increase in TBARS and reduction in GSH levels. Pretreatment with combination of celecoxib with duloxetine (5, 10 mg/kg) showed significant antidepressant and neuroprotective effects against stress induced depression and oxidative damage in mice at both dose levels.

Conclusions: This study demonstrated dose-dependent antidepressant action of celecoxib in stressed mice. The combination of celecoxib with duloxetine further enhanced its antidepressant effect on TST in stressed mice. The treatment reversed forced swim-induced elevation in TBARS levels and depleted glutathione activity, suggesting their antioxidant and protective role in brain.

SL-89

Track: *Drug Discovery in Preclinical Research*

TUMOR LUNG CANCER MODEL FOR ASSESSING ANTI-NEOPLASTIC EFFECT OF PMF IN RODENTS: HISTOPATHOLOGICAL STUDY

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This study aims to elaborate an experimental model of pulmonary carcinogenesis to ascertain the preventive efficacy of PMF as anticancer drug. Mice were used through an intra-peritoneal injection of the Benzo[a] pyrene for tumoral lung induction. Four experimental groups had been used: control group; carcinogenic group without treatment, treated carcinogenic group and the positive control group that received only treatment. Each submitted to euthanasia 08, 16, 24 weeks after the experimental procedure. After 08 weeks, the presence of diffuse inflammatory alterations was observed in carcinogenic- non treated group with thickness of the alveolar wall after the inflammation, however, at analysis of the pulmonary tissue of the treated carcinogenic group it had been observed hyperplasic alterations (BALT hyperplasia), but in positive control group thickness of the alveolar wall was noticed. With more time, after 16, 24 weeks administration of PMF histopathological changes became lesser in the treated carcinogenic group as compared to animals treated with the B[a]P only.

In conclusion, the main secondary alterations in the intra-pulmonary instillation of B[a]P were: cellular proliferation, inflammatory alterations of several degrees. PMF treatment has a slightly protective effect to lung tissue along short time, but with more time it improved the structure of the lung in carcinogenic treated group.

Keyword: Benzo[a] pyrene, PMF, A549 cell line, BALT hyperplasia.

SL-138

Track: *Pharmaceutical Research & Development*

AN OINTMENT CONTAIN 3% OF PMF COMPONENTS SHOWED PROMISING RESULTS FOR TREATMENT OF PSORIASIS

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Background: Previous investigations of PM 701 which is natural product sterile and free from toxicity (Khorshid *et al.*, 2005; Khorshid and Mosherf, 2006; Mosherf *et al.*, 2006; Khorshid, 2008) proved its reparative effect on normal human cell (foreskin).

The whole study involved formulation of PM 701 as a powder, and investigate the efficiency of pharmaceutical formulation on the normal skin cells in tissue culture. Which had proved its efficiency in nourishing fibroblast skin cells; also the previous study includes preclinical experiments that addressed the toxicological effect on various animal species (Khorshid, 2008). The toxicological studies were done on formulated PM701. That experiments proved their safety as indicated by the high value of MTD on animal models and also by its safety effect on vital organs of animal models through histopathological studies carried out using light and electron microcopies.

PM701 had been separated into six parts and assess the impact of each part separately on the normal fibroblast cells in cultures of human skin. The fraction, which was coded PMF (150mg/g of the lyophilized PM701) was able significantly to nourishing the normal skin cells, while the other five fractions were less effective (Khorshid *et al.*, 2009; Khorshid, 2009). PMF was formulated as an ointment containing 3 % of PMF in oleaginous base. These formulations are strong candidate for further studies and researches as long as experiments had shown the capacity of it to treat many skin diseases.

SL-90

Track: *"Anti-Cancer Drug Discovery & Therapy"*

INDUCTION OF TUMOR CELL DEATH BY SURVIVIN NUCLEAR EXPORT INHIBITORS

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Survivin's dual role as an apoptosis inhibitor and a mitotic effector positioned this smallest member of the inhibitor of apoptosis protein (IAP) family in the front line of cancer research. Survivin plays an essential role in mediating resistance to chemo- and radiation-therapy and thus, has direct clinical relevance. Recent evidence demonstrated that Survivin's interaction with the nuclear export receptor Crm1 is critically involved in its intracellular localization and cancer relevant functions. Hence, interference with Survivin's nuclear export by chemical decoys is considered a promising strategy to counteract therapy resistance. We engineered autofluorescent cellular translocation biosensors, and used the Cellomics®ArrayScan high-throughput microscopy platform to screen 17,000 small molecules for cell-active Survivin export inhibitors. Identified bioactive compounds not only reversibly but also irreversibly blocked Survivin's nuclear export at µM concentrations in several liquid and solid tumor cell lines. Export inhibition resulted in p53-independent apoptosis, also in primary human head and neck tumor cells. In contrast, primary non-malignant human cells were not affected and no signs of toxicity were seen upon injection into nude mice.

Besides their relevance as chemical tools to further dissect the tumor-promoting (patho)biological functions of Survivin these inhibitors might be further developed into novel anti-cancer drugs.

Keywords: Nucleocytoplasmic transport, chemical biology, apoptosis, therapy resistance.

SL-162

Track: *Enabling Technologies*

MODULAR CONSTRUCTION OF APTAMERIC NANOSTRUCTURES: ANTI-THROMBIN APTAMERS

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Two conventional paradigms in drug design are Chemicals and Biologics. Fast growing third novel paradigm is Nucleic Acid Aptamers which are promising alternative to antibodies in theranostics. Several DNA aptamers against human thrombin (TBAs) have been developed by combinatorial library selection (SELEX), though no direct structure based drug design for the aptamers has been performed yet.

Structural data on the basic DNA aptamer, G-quadruplex 15mer, both along and in complex with thrombin, are rather controversial. Improved force field for computer molecular dynamics simulations has been developed, allowing to solve ambiguities, to make models of known modular aptamers (31mer, NU172), and to design a novel modular DNA aptamer, RA36, with improved characteristics. By UV melting, CD and capillary electrophoresis, conformational transitions as well as the module influence within the aptamers have been studied (N Dolinnaya collaboration). SPR and SPRi affinity measuring were performed (J Zhu collaboration). A newly developed thrombin activity assay, as well as specific kinetics calculations, has been developed. RA36 and 31TBA are effective at much lower concentrations (5 - 20 nM) than 15-TBA. Types of inhibiting are also vary. RA36 is covered with patents, and currently under pre-clinical trials. Supported by Ministry of Education and Science of RF 16.512.11.2009.

Keywords: Anti-trombin, aptamer.

SL-190

Track: *Anti-Infectives*

INTERFACE INHIBITORS OF MACROMOLECULAR INTERACTIONS – RIBOSOMAL PARADIGM FOR CREATION OF NOVEL ANTIBIOTICS

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Most of anti-bacterial agents inhibit enzyme activities, including giant one like ribosome. Due to fast progress of interactomics novel pharmaceutical trend has emerged – development of protein-protein and protein-nucleic acid interaction inhibitors for intervention into key cellular processes, thus inhibiting not final complex structure functioning but structure's biogenesis/assembly.

Ribosome is paradigm for many structure-function studies of antibiotic. Translational antibiotics block ribosomal active centers including codon-anticodon interactions, peptidyl transferase, translational factor functioning. On the other hand, ribosome biogenesis is the most expensive cellular process consuming more than half of bacterial resources. Therefore study of ribosomal biogenesis as target for creation of novel antibiotics has great value.

The report revealed mechanisms of bacterial ribosomal small subunit (RSS) biogenesis. Out of 20 RSS proteins, two are the key ones for biogenesis – S4 and S7. During fast bacterial cell growth S4 and S7 determine subunit assembly. During slow bacterial cell growth these proteins interact with their mRNA (S7 – with streptomycin mRNA, str mRNA), and inhibit its own translation, and hence – ribosome assembly. Complexes of S7 with both fragments – 16S rRNA and str mRNA, has been studied in details for three microorganisms: *E. coli, T. thermophilus, S. coelicolor*. Work is supported by RFBR 11-04-01990-a.

Keywords: streptomycin, ribosome, antibiotics, RNA-protein interactions.

SL-193

Track: *Pharmaceutical Biotechnology*

BIOSYNTHESIS AND DIVERSIFICATION OF LIPOGLYCOPEPTIDE ANTIBIOTICS

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The emergence of bacterial resistance to clinically used antibiotics threatens lives. New generations of drugs with better efficacy thus are urgently demanded. Countless efforts have been made in searching for new drugable chemical entities, while the thirsty for them is much more than it was. For this concern, diversity-oriented biosynthesis for privileged natural products appears as a new direction for drug development.

The privileged compounds here are clinically important lipoglycopeptide antibiotics teicoplanin and A40926. A lipo-sugar moiety in these compounds plays a pivotal role in drug effectiveness. The biosynthetic pathway for this lipo-sugar moiety has been elucidated. In this study, the function of deacylase (Orf2) in the pathway was expanded on the basis of ligand-bound deacylase crystal structures. With new facets of Orf2 and other enzymes, we were in a position redirecting the synthetic pathway towards new compounds (e.g. reposition of N-acyl Glm from one residue to another).

In addition, crystal structures of Dbv29 in the pathway free and in complex with an intermediate were also revealed that an aldehyde group of the intermediate is exposed to solution and susceptible to derivatization. Of the structural information, a one-pot chemo-enzymatic synthesis was developed to generate new analogs of teicoplanin/A40926, some of which showed marked potency and efficacy against multi-drug resistant pathogens.

SL-197

ASSOCIATION OF ETOPOSIDE TO CHOLESTEROL-RICH NANOEMULSIONS REDUCES ATHEROSCLEROTIC LESIONS IN RABBITS: A NOVEL DRUG TARGETING-BASED STRATEGY FOR THE TREATMENT OF CARDIOVASCULAR DISEASE

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Atherosclerosis is basically a chronic inflammatory process but the current clinical treatment of the disease is restricted to control of risk factors. Previously, it was shown that cholesterol-rich nanoemulsions (LDE) bind to LDL receptors and after injection into the bloodstream concentrate in aortas of atherosclerotic rabbits. Thus, LDE may carry drugs targeted to atheromas. The aim was to verify the ability of etoposide, a potent antiproliferative agent used in cancer treatment, to reduce lesions and inflammatory factors in atherosclerotic rabbits. Eigthteen rabbits were fed 1% cholesterol for 60 days. After 30 days, nine animals were treated with four weekly intravenous injections of etoposide-oleate (6 mg/kg) associated with LDE for comparison with nine control animals. LDE-etoposide reduced the lesion areas by 85% and intimal width by 50% . Macrophage and smooth muscle cell invasion of the intima was impaired. LDE-etoposide also markedly reduced the protein expression of inflammatory cytokines (IL-1β, TNF-α), MMP9 and cell proliferation markers (topoisomerase IIα, tubulin). Toxicity was not observed. The ability of LDE-etoposide to strongly reduce the lesion area and the inflammatory process of atherosclerosis without toxicity warrants the great therapeutic potential of this novel pharmaceutical formulation to target the inflammatory-proliferative basic mechanisms of the disease.

Keywords: Nanoparticles, drug delivery, etoposide, atherosclerosis treatment, cholesterol.

SL-76

Track: *Cardiovascular Drug Discovery & Therapy*

EFFECTS OF NEFERINE ON PLATELET AGGREGATION AND DISAGGREGATION

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Neferine (Nef), a kind of isoquinoline alkaloid, extracts from the seed embryo of *Nelumbo nucifera Gaertn*. Previous studies have shown that neferine has a wide range of biological activities. However, it is unknown whether neferine also has an effect on platelet activity. This study investigated the antiplatelet properties of this natural compound. Neferine dose-dependently inhibited mouse platelet aggregation induced by collagen related peptide (CRP), collagen, ADP, thrombin and U46619. At 3 μM, neferine effectively inhibited the maximal extent of aggregation by CRP as much as 99.5%. It also dramatically inhibited the phosphorylation of Akt(Ser473) and Src(Y416), however, no effect was found in the phosphorylation of ERK. It also activation-depended phosphorylated VASP on Ser157, but did not affect the PKC and FAK. Interestingly, platelet aggregation induced by CRP was significantly disaggregated by 3 μM Neferine , which reached 44.4%. And neferine disaggregated ADP induced platelet aggregation in a concentration-dependent manner. The phosphorylation of Akt(Ser473) was also dramatically inhibited, but not the same result with ERK. We conclude that neferine inhibits platelet activation induced by GPVI agonists through interfering with the kinase activity of Src(Y416), activing VASP (Ser157) phosphorylation and the subsequent activation of PI3K-Akt pathway. Meanwhile, it also disaggregated CRP and ADP-mediated platelet aggregation.

SL-210

Track: *Protein & Peptide Sciences*

ISOLATION OF 62 kDa PROTEIN WITH ANTIOXIDANT PROPERTIES FROM NATURAL HONEY

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It is strictly evidence that honey contains antioxidant activity; however, constituents of honey responsible for its antioxidant activity are still needed to be investigated. The aim of this study w as to purify protein fraction on the basis of antioxidant activity. Fourteen natural honey samples from North Africa and South Asia were evaluated for their antioxidant activities by employing 1, 1 -Diphenyl-2 - picrylhydrazyl (DPPH) radical assay. The scavenging activity of all honey samples ranged 18.7-32.6 %. A 62 kDa protein isolated from honey by gel filtration chromatography, separated by SDS-PAGE and further purified by reverse phase HPLC has shown an increased (54.6 %) scavenging activity, this indicates the possible role of this protein fraction in the antioxidative and therapeutic properties of the natural honey.

Keywords: Honey characterization; antioxidant; apitherapy.

SL-17

Track: *Hot Topics in Natural Product*

INHIBITORY ACTIVITY OF CINNAMON ZEYLANICUM AND EUCALYPTUS GLOBULUS OILS ON *STREPTOCOCCUS MUTANS, STAPHYLOCOCCUS AUREUS*, AND CANDIDA SPECIES ISOLATED FROM PATIENTS WITH ORAL INFECTIONS

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Statement of Problem: The *Strep. mutans, Staph. aureus, Cand. albicans,* and *Cand. glabrata* are the most common cuases of oral infections and the use of chemical drugs usually leads to drug resistant microorganisms.

Purpose: The aim of this study was to investigate the antimicrobial activity of Cinnamon and Eucalyptus oils against this oral microorganism.

Materials and Methods: The oils were prepared by steam distillation and their inhibitory activity at different concentrations and the minimum inhibitory concentrations (MIC) were determined. *Strep. mutans*, multi drug resistant (MDR) *Strep. mutans, Staph. aureus*, methicilin resistant *Staph. aureus, Candida albicans, C. glabrata* were used in this study. The data were analyzed using Chi square and Fisher statistical test.

Results: All of the bacterial and fungal isolates were sensitive to Cinnamon and Eucalyptus. Cinnamon oil showed strong promising inhibitory activity on all the *S. mutans* isolates at a concentration as low as 3.12%. Eucalyptus oil

showed less inhibitory activity, as the least effective concentration of this oil was 25%. The MIC of Cinnamon and Eucalyptus oil ranged 12.8-51.2 and 64-256mg/ml, respectively.

Conclusion: Both Cinnamon and Eucalyptus oils exhibited antimicrobial activity but their effectiveness varied. Cinnamon oil showed stronger inhibitory activity as measured by MIC determination. *S. mutans*, the etiological agents of dental caries, were highly sensitive to Cinnamon oil and hence it may be used as an antiseptic in toothpaste, mouthwash or chewing gum for prevention of dental caries and other oral infections.

Keywords: Cinnamon, Eucalyptus, *S. mutans, S. aureus,* MIC.

SL-20

Track: *Medical Imaging*

CLINICAL VALUE OF MULTIFOCAL-ELECTRORETINOGRAM (mf-ERG) IN RETINAL DISEASES

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Multifocal-ERG (mf-ERG) system can stimulate multiple retinal areas simultaneously and detect each response independently. With this method we have succeeded in constructing an ERG topography of fine resolution, which allows a functional mapping of the retina and indicates not only the central loss of function in maculopathies but also allows a detailed description of the extent of the lesion.

The reduction of central retinal activity can be detected in all kinds of maculopathies as in Stargardt disease, in cone dystrophy, Vitelliform maculopathies, juvenile retinoschisis, macular hole, epiretinal membrane, central serous chorioretinopathy, age-related macular degeneration (AMD), diabetic retinopathy, central retinal vein occlusion (CRVO), and others.

Since some years the anti-VEGF like bevacizumab, ranibizumab or Pegaptanib are used widely for choroidal neovascularization due to AMD, CRVO or diabetic maculopathy. For monitoring the evolution of these diseases, the study of electrical activity of the macula with mf-ERG may be more reliable for the assessment of macular function.

In conclusion the mf-ERG is a reliable objective diagnostic method not only for the detection and evaluation of macular function even in eyes without clinical symptoms, but also to monitor the evolution of a macular disease treated surgically or pharmaceutically.

SL-106

Track: *Recent Advances in Patient Treatment and Care*

INTENSITY OF COMBINED MODALITY CHEMOTHERAPY AND PET-GUIDED RADIOTHERAPY IN PATIENTS WITH ADVANCED STAGE HODGKIN LYMPHOMA

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BACKGROUND: The intensity of chemotherapy and need for additional radiotherapy in patients with advanced stage Hodgkin lymphoma (HL) is unclear. We thus conducted a prospective randomized clinical trial in a three arm parallel group design to demonstrate the non-inferiority of two less intensive experimental chemotherapy regimens, followed by positron emission tomography (PET) guided consolidating radiotherapy.

METHODS: Patients with newly diagnosed advanced stage HL were randomly assigned to either eight cycles of BEACOPPescalated (8Besc), six cycles of BEACOPPescalated (6Besc), or eight cycles of BEACOPP14 (8B14).

After finishing of chemotherapy, the complete imaging - including PET, CT and MRI - of every individual patient were centrally reviewed by a multidisciplinary panel in Cologne.

Patients with a persistent mass, measuring 2.5 cm or more, and positive on FDG-PET scan after chemotherapy received additional radiotherapy (RT) with 30 Gy to the persistent mass.

The main endpoint of this study was freedom from treatment failure (FFTF); other endpoints included overall survival, tumor response, side effects of treatment and the negative predictive value (NPV) of PET with regard to the need of consolidating radiotherapy on persisting PET positive masses.

RESULTS: A total of 2,182 patients were randomized among the three study arms. FFTF at 5 years was 84.4% in the 8Besc group, 89.3% in the 6Besc group (97.5%; CI for difference between 8Besc and 6Besc 0.5% to 9.3%), and 85.4% in the 8B14 group. OS was 91.9%, 95.3%, and 94.5%, and was also better with 6Besc compared with 8Besc (97.5%; CI 0.2% to 6.5%).

728 pts. with residual masses of ≥ 2.5 cm after chemotherapy were evaluated by PET, 188 pts. were PET+, 180 received radiotherapy. At 12 months 11 % events occurred in PET+ pts., compared with 4 % in PET- pts.

The NPV for PET at 12 months was 94.1% (95%; CI 92.1% to 96.1%) and only 11% of all patients received additional RT.

CONCLUSION: Treatment with 6 cycles of BEACOPPescalated followed by PET-guided RT is more effective and less toxic than 8 cycles of BEACOPPescalated in patients with advanced stage Hodgkin Lymphoma.

FDG-PET, performed after chemotherapy, can guide the necessity of additional RT in this setting. Only those advanced-stage Hodgkin Lymphoma patients with residual disease and are PET+ might need consolidating radiotherapy.

Keywords: Lymphoma, combined modalty well-directed treatment, Intesity of chemotherapy, consolidating radiotherapy.

SL-195

Track: *Anti-Infectives*

FULVIC ACIDS AND VIRAL INFECTIONS

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Active principles in humic substances are fulvic acids, which promote better absorption of mineral ions from soil by vegetables. They are formed by large organic molecules with several reactive sites. Tested in mice and rats with radioactive labeled ions ($^{45}Ca^{2+}$, $^{32}PO_4^{3-}$, $^{59}Fe^{3+}$ and $^{131}I^-$) in drinking water, it has been found that for same concentrations of mineral ions (μg/ml), more than double are filtered through liver from stomach to blood serum if calcium and phosphate ions are escorted by fulvic acids, while iron and iodine ions are completely fixed by blood (red cells) and thyroid gland respectively, also in a proportion larger than double when fulvic acids are present. This paper presents the associated effect of fulvic acids ingestion by rats and mice, increasing substantially their total and G immunoglobulins (TIg, GIg) production, which explains the empirical and preliminary results, but by no means less spectacular results obtained to prevent epidemic viral infections in cattle and poultry, as well as an effective agent in the treatment of human viral infections such as herpes Zoster, hepatitis and HIV. As a conclusion, this scarce and partial results suggest the necessity to initiate a large, serious and quite reliable medical protocol, which at present has not been possible.

SL-35(a)

Track: *Hot Topics in Natural Products*

CYTOTOXICITY OF ANTIMALARIAL PLANT EXTRACTS FROM KENYAN BIODIVERSITY TO THE BRINE SHRIMP, *ARTEMIA SALINA L.* (ARTEMIIDAE)

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Background: *Artemia salina* (Artemiidae), the brine shrimp larva, is an invertebrate used in the alternative test to determine toxicity of chemicals and natural products.

Design and methods: In this study the Medium Lethal Concentrations (LC_{50} values) of 45 antimalarial plant extracts and positive controls, cyclophosphamide and etoposide were determined using *Artemia salina* (Artemiidae).

Results: Out of the 45 organic extracts screened for activity against *Artemia salina* larvae, 23 (51%) of the crude extracts demonstrated activity at or below 100 µg/ml, and were categorized as having strong cytotoxic activity, 18 (40%) of the crude extracts had LC_{50} values between 100 µg/ml and 500 µg/ml, and were categorized as having moderate cytotoxicity, 2 (4.5%) of the crude extracts had LC_{50} values between 500 µg/ml and 1000 µg/ml, and were considered to have weak cytotoxic activity, while 2 (4.5%) of the crude extracts had LC_{50} values greater than 1000 µg/ml and were considered to be non toxic. Approximately 20% (9) of the aqueous extracts demonstrated activity at or below 100 µg/ml and were considered to have strong cytotoxic activity, 40% (18) of the screened aqueous crude extracts had LC_{50} values between 100 µg/ml and 500 µg/ml and were considered to be moderately cytotoxic, 16% (7) of the crude extracts had LC_{50} values between 500 µg/ml and 1000 µg/ml and were considered to have weak cytotoxic activity while 24% (11) of the aqueous extracts had LC_{50} values greater than 1000 µg/ml and were categorized as non toxic.

The positive controls, cyclophosphamide and etoposide exhibited strong cytotoxicity with LC_{50} values of 95 µg/ml and 6 µg/ml respectively in a 24 hour lethality study, validating their use as anticancer agents.

Conclusions: In the current study, 95.5% of all the screened organic extracts and 76% of the investigated aqueous extracts demonstrated LC_{50} values <1000 µg/ml, indicating that these plants could not make safe antimalarial treatments. This calls for dose adjustment amongst the community using the plant extracts for the treatment of malaria and chemical investigation for isolation of bioactive compounds responsible for the observed toxicity. These could make novel ingredients for anticancerous drugs.

Keywords: Cytotoxicity, *Artemia salina* bioassay, crude extracts, antimalarial plants, Kenyan biodiversity.

SL-71

Track: *Drug Discovery in Preclinical Research*

HOW DO MACROLIDE ANTIBIOTICS INTERACT WITH BACTERIAL RIBOSOMES?

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Macrolide antibiotics, such as azithromycin, have been widely prescribed for the treatment of respiratory tract infections owing to their high efficacy and safety. In spite of a number of existing macrolide antibiotics, the emerging multi-drug resistant microbial pathogens present serious and challenging problems which demand novel and more effective antimicrobial agents to be discovered.

An effective approach to overcoming this problem is to understand the principles of how these drugs interact with their target ribosome. Recently available crystal structures of some ribosome-macrolide complexes have thrown new light on the binding mechanisms. However, when analysing solid state structures of ribosome-macrolide complexes one should keep in mind the discrepancies between obtained results. We believe that important steps in drug design should also include elucidation of the solution-state structures of free and bound macrolides since the structural features of the complex may not be exactly the same in solution and in the solid state.

Here, a combination of NMR spectroscopy and molecular modeling is employed to determine free and bound conformations of macrolides and their binding epitopes in solution state. The knowledge gained from these studies will be discussed which can help in developing strategies aiming at design of potential inhibitors.

Keywords: Antibiotics, bacterial, macrolide, ribosomes.

SL-88

Track: *Cardiovascular Drug Discovery & Therapy*

KNOWLEDGE OF DISEASE AND ADHERENCE TO DRUG THERAPY IN PERSONS WITH TYPE 2 DIABETES AND HYPERTENSION

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This study was carried out to determine the level of knowledge of disease and adherence to drug therapy among patients with Type 2 diabetes and Hypertension. One hundred and seventy-seven (177) patients attending cardiology and endocrinology clinics at University College Hospital (UCH) Ibadan, in Nigeria participated in the study. Socio-demographic characteristics, patients' knowledge of diabetes and hypertension and adherence to drug therapy were determined with the use of a pre-tested questionnaires. Anthropometric measurements and blood pressure were taken with fasting blood glucose.

Exactly 45.2% of the study population were males and 54.8% were females. Patients who had Type 2 diabetes alone were 23.0% while 20.3% had hypertension alone and 55.9% had type 2 diabetes with hypertension. The mean age was 63.2 ± 10.7 years. Exactly 64.0% of the study population had good knowledge of diabetes and hypertension while 58.8% were adherent to drug therapy by self-reported methods. Blood pressure control was poor among patients who had hypertension as only 42.9% of hypertensives had a systolic blood pressure of less than 140mmHg and diastolic blood pressure of less than 90mmHg. Patients' with Type 2 diabetes with hypertension blood pressure control was poor because only 41.9% had a systolic blood pressure of < 130mmHg and a diastolic blood pressure of < 80mmHg. Exactly 36.0% of patients with Type 2 diabetes alone and 40% of patients with type 2 diabetes with hypertension had good glycaemia control. Descriptive statistics, Chi-square tests and Pearson correlation were used in evaluating the data obtained.

Knowledge of diabetes and hypertension among these patients was above average however adherence to drug therapy was poor hence treatment goals were not achieved. This calls for increase in awareness of the importance of adherence to drug therapy among patients with type 2 diabetes and hypertension which will involve a concerted effort by all members of the healthcare team.

Keywords: Type 2 Diabetes mellitus, Hypertension, Adherence, Knowledge.

SL-140

Track: *CNS Drug Discovery & Therapy*

A SURVEY OF RATIONAL PRESCRIPTIONS OF PENICILLIN AND CEPHALOSPORIN ANTIBIOTICS IN SACRED HEART HOSPITAL, LANTORO ABEOKUTA IN SOUTH WEST NIGERIA

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This study examines the pattern of prescription of penicillins and cephalosporins in a secondary mission hospital in Nigeria.It was a retrospective study involving data obtained from outpatient case notes that were prescribed with cephalosporins and penicillins from January to June 2010. A total of six hundred and fourteen (614) case notes were randomly selected and used for the study.

One hundred and sixty seven patients (167) (27%) were of age group 0 - 9 years, (31)(5%) were 10 – 19 years and 226(37%) were of age group 20 – 29 years . Four hundred and fourty (440) (71.7%) were males and 174 (28.3%) were females. Diagnosis made included upper respiratory tract infection (URTI) 238 (38.8%) and lower respiratory tract infection (LRTI) (21.0%) P = 0.00393. No definite diagnosis (NDD) was made in 37 (6.02%) of the cases.

Penicillins were prescribed for 474 (77.2%) and Cephalosporins for 140 (22.8%) patients.Both classes of antibiotics were prescribed most frequently for URTI. The cost of filling a prescription follows a normal curve distribution with the peak at the age group 35 – 49 years for the penicillins but the cost decreased with increasing age for the Cephalosporins. Generic prescriptions were found to be 198(32.2%) and proprietary prescriptions were 416(67.8%). Mean duration of

prescription for penicillins was 6.65 + 1.95 days while it was 5.5 + 1.5 days for the cephalosporins. There were no documented cases of microbial culture sensitivity test (MCS) and adverse effect.

The prescription pattern of penicillins and cephalosporins seemed not to be in line with standard guidelines of antibiotics therapy because adequate measures appeared not taken to detect and document adverse drug reactions and microbial culture sensitivity test.

Keywords; Penicillin Antibiotics, Cephalosphorin Antibiotics, Rational Drug Prescription, South West Nigeria.

SL-22

Track: *CNS Drug Discovery & Therapy*

SYSTEMATIC OVERVIEW OF COCHRANE REVIEWS FOR ANTICHOLINERGIC EFFECTS OF ANTIPSYCHOTIC DRUGS

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Background: Despite much being written on the topic, there are few surveys investigating the prevalence of anticholinergic adverse effects of antipsychotic drugs. One study, however, used trial-derived data to calculate estimates.

Objectives: To investigate the prevalence/incidence rates of anticholinergic effects as viewed from within relevant randomized trials.

Methods: Data were extracted from each relevant study included in Cochrane reviews. Data were checked, extracted, and simple frequencies, and 95% confidence intervals (CIs) were calculated.

Results: Many trials in relevant reviews reported no data on anticholinergic effects (estimate 40,000 participants). However, data were extracted from 177 studies within 54 reviews (N = 27,328 participants). Most data are short-term (<12 weeks). For blurred vision, the newer generations of drugs have rates of between 10% and 20%. These estimates are similar to those of sulpiride and chlorpromazine, less than trifluoperazine, but considerably more than perphenazine. Data are presented on a range of anticholinergic effects across different periods.

Conclusions: Anticholinergic symptoms are common adverse effects associated with the use of all antipsychotic drugs, and newer-generation drugs are not clearly distinguishable from many older compounds. Adverse effect data should be more accessible.

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SL-124

Track: *Recent Advances in Patient Treatment and Care*

SYSTEMATIC REVIEW AND META-ANALYSIS OF ANTICHOLINERGIC SIDE EFFECTS OF LONG-ACTING ANTIPSYCHOTICS

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Background: There are not many studies on anticholinergic side effects of long-acting antipscyotics. They tend to be used with stigmatized, severely ill and non-compliant patients rather than first onset psychosis.

Aim: To investigate prevalence/incidence rate of anticholinergic side effects of long-acting antipscyotics.

Methods: We included all participants with schizophrenia, schizoaffective disorder or schizotypal disorder on depot antipsychotics in the trials within all Cochrane reviews published by Cochrane schizophrenia group. One researcher searched the Cochrane library and extracted data into Microsoft Excel to analyze frequencies, prevalence and confidence intervals of the various anticholinergic side effects of all the long acting antipsychotic depots found.

Result: We found seven reviews for seven depot antipsychotics. For example for Fluphenazine decanoate at least a quarter of the participants experienced blurred vision 24.5% (CI 11 to 47) in the short-term, 16 % (CI 10-27) in the medium-term, and 21.4 % (CI 16-28) in the long-term.

Conclusion: The disturbing anticholinergic side effects of depot antipsychotics are not any more frequent than the anticholinergic side effects of oral antipsychotics. There is no evidence to suggest that oral medications are better tolerated than long-acting depot preparations.

SL-19

Track: *Anti-Cancer Drug Discovery & Therapy*

EPOXY-EICOSANOIDS STIMULATE MULTI-ORGAN METASTASIS AND TUMOR DORMANCY ESCAPE

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Endogenously-produced lipid autacoids are locally-acting small molecule mediators that play a central role in inflammation and in the response to tissue injury. Although these autacoids, including prostaglandins and leukotrienes, are best known as products of arachidonic acid metabolism by cyclooxygenases and lipoxygenases, arachidonic acid is also a substrate for the cytochrome P450 epoxygenases which convert it to epoxyeicosatrienoic acids (EETs). The bioactive EETs are produced predominantly in the endothelium and are metabolized by soluble epoxide hydrolase (sEH) to less active dihydroxyeicosatrienoic acids (DHETs). EETs regulate inflammation and vascular tone. Despite pleiotropic effects on cells, little is known about the role of these epoxyeicosanoids in cancer. Since EET levels are directly influenced by nutrients and inflammatory processes, we investigated the effect of endothelial-derived and systemic EETs on cancer in a variety of animal models. Using genetic and pharmacological manipulation of endogenous EET levels we demonstrate that EETs are critical for primary tumor growth and metastasis. EETs promoted tumor growth independent of the specific tumor model used, ranging from subcutaneous and orthotopic transplantable to spontaneous tumors. Upregulation of EET production in endothelial cells leads to increased angiogenesis, extensive multi-organ metastasis, and escape from tumor dormancy. Below a critical threshold of tumor cell number, EETs are necessary for normal tumor development. By manipulating EET levels *in vivo* we demonstrated the dramatic metastatic potential of EETs on low-metastasizing tumors. Parabiosis models show that this systemic metastasis was not caused by excessive primary tumor growth but depended on endothelial EETs at the site of metastasis. Administration of synthetic EETs recapitulated these results while EET antagonists suppressed tumor growth and metastasis, providing the first *in vivo* demonstration that pharmacological modulation of EETs can affect cancer growth. Our data suggests that the increase of EETs, due to down-regulation of sEH may support the angiogenic switch by an increase of VEGFR2 and loss of thrombospondin. We also demonstrate that sEH inhibitors, which elevate endogenous EET levels, promote primary tumor growth and metastasis. These findings are of pivotal clinical relevance since drugs that raise EETs are in clinical trials for the treatment of hypertension. We also demonstrate that specific EET antagonists inhibit primary tumor growth and metastasis. EET production in the endothelium may be a critical regulator of tumor growth and metastasis, offering a mechanistic rationale for using EET antagonists as novel anti-cancer therapeutics. Our results could pave the path for a new strategy for the prevention and treatment of metastatic disease – i.e. inhibition of EET bioactivity. Specific EET antagonists, inhibitors of endothelial CYP epoxygenases, or the overexpression of EET metabolizing enzymes may represent new strategies for the treatment of cancer. These observations indicate a central role for EETs in tumorigenesis, offering a mechanistic link between lipid autacoids and cancer.

SL-191

Track: *Regenerative Medicine*

EPOXY-EICOSANOIDS PROMOTE TISSUE AND ORGAN REGENERATION

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Epoxyeicosatrienoic acids (EETs) are lipid mediators produced by cytochrome P450 epoxygenases that regulate inflammation, angiogenesis and vascular tone. Despite pleiotropic effects on cells, the role of these epoxyeicosanoids in normal tissue and organ regeneration remains unknown. EETs are produced predominantly in the endothelium. Normal tissue and organ regeneration require an active role of the microvascular endothelium, a process known to be dependent on angiogenic growth factors. Thus, we hypothesize that endothelial cells (ECs) stimulate tissue and organ regeneration via production of bioactive EETs. To determine whether endothelial-derived EETs affect physiologic tissue growth *in vivo*, we utilized genetic and pharmacological tools to manipulate endogenous EET levels. We show that endothelial-derived EETs play a critical role in accelerating tissue growth *in vivo* including wound healing, corneal neovascularization, retinal vascularization, liver regeneration, kidney regeneration and lung regeneration. Administration of synthetic EETs recapitulated these results while lowering EET levels either genetically or pharmacologically delayed tissue regeneration, providing *in vivo* demonstration that pharmacological modulation of EETs can affect normal tissue growth. We also demonstrate that sEH inhibitors, which elevate endogenous EET levels, promote liver and lung regeneration. Thus, our observations indicate a central role for EETs in tissue and organ regeneration, and their contribution to tissue homeostasis.

SL-27

Track: *Regenerative Medicine*

INDUCTION OF NEURAL DIFFERENTIATION OF MAMMALIAN ADIPOSE STEM CELLS (ASCs), AND THE CELL BEHAVIOR AFTER TRANSPLANTATION INTO MOUSE BRAIN

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Our studies showed that ASCs incubated in medium containing BDNF and 5-azacytidine as induction factors gained neural features. Neural induction of ASCs displayed expression of 4 neural genes eno2, map2, beta-3-tub, and nestin. The induction effect of BDNF was mediated by the receptor TrkB, which mRNA was detected in the initial ASCs population. In our experiments ASC expressing green fluorescent protein, were subjected to the inductive effects of BDNF and 5-azacitidine, within 3 days. Then these cells were injected into striatum of Black6 mice. Through 7, 9 and 11 days the distribution of transplanted cells on sections of the brain was analyzed. After 9 days, many induced ASCs migrated into the surrounding brain parenchyma. Control uninduced cells were arranged locally and did not extend beyond the injection site. By the 11th day after injection the control cells disappeared from the brain sections, whereas induced cells remained alive. The data obtained suggest that the effect of BDNF with the background of 5-azacytidine not only promotes neural differentiation of ASCs and increases the expression of neural genes, but also increases the survival of cells after transplantation into brain tissue. Immunohistochemical staining of sections containing the transplanted cells showed that induced by the developed method ASCs expressed dablkortin. Transplantation of induced ASCs had stimulated a migration of the host neuroblasts toward the site of injection.

The preliminary experiments on transplantation of induced cells into the mouse striatum after Endotelin I injection had shown that in the ischemic brain they behaved very actively. In the former case the active migration of the induced cells into brain parenchyma occurred. The cells migrated mainly along the blood vessels, and there are also some free migrating cells. The induced cells survived in the ischemic brain up to the 14 days while uninduced cells were completely dead at that time.

SL-108

Track: *Proteomics and Bioinformatics*

DOMAIN FUSIONS IN RESTRICTION-MODIFICATION SYSTEM ENZYMES AND GENOMIC CONTEXTS OF FUSIONS

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Consecutive steps in reaction chains are often executed by different compone nts of protein complexes. Sometimes the genes of individual components become fused enabling reaction steps to be performed more efficiently through direct handover of the product to the next enzymatic center. Sometimes, gene fusions of apparently unrelated enzymes are found, which may reflect chance events or may indicate some new biology is taking place. Surprisingly, we have recently found examples of fusions between a restriction enzyme and its cognate methyltransferase (usually these are separate and independent enzymes). In several cases these have been demonstrated to act as typical restriction-modification (RM) enzyme genes that shield their hosts against phage invasion.

Restriction enzymes' ability to cleave DNA precisely makes them crucial DNA engineering and genome interrogation tools - this lends additional importance to the study of these enzymes. We analyze the restriction enzyme sequence collection of the curated REBASE resource. In our paper we will describe algorithms designed to detect domain shuffling, gene duplication, and recruitment of non-RM domains that couple DNA unwinding, looping, and chemical modification functionalities to site specific recognition. Interpretation of the findings against full genome contexts also opens the possibility for the identification of stand-alone restriction enzymes.

SL-120

Track: *Translational Medicine*

DEVELOPMENTAL SELF ORGANIZATION AND AUTISM - A HYPOTHESIS

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Brain development is an input driven self-organizing process. Sensory stimulus is critical for development, later functional performance reflects on developmental patterns, and inappropriate input manifests in dysfunction (e.g. amblyopia). I argue that other functional disorders of the brain, particularly autism, result from inappropriate developmental inputs as well.

Autism is a psychological disorder, characterized by difficulties in communication and social interaction. Prevalence figures are around 1 in 140, and diagnosis is typically established around age two. A multitude of possible causes have been explored, yet none has produced significant results in treatment, cure or prevention.

Brain regions mature gradually. Synapses stabilize under the steady flow of sensory inputs, as neural circuits learn to recognize patterns in those inputs. Peak synaptic connectivity is reached around age two. Neural circuits process whatever input they receive, even non-evolutionary signals. However, at the end, non-evolutionary inputs may cause dysfunction, because newly formed processing circuits are not time-tested. They may interfere with other modules, occupy neural bundles needed for other functions, consume energy or dissipate heat in harmful excess. Atypical neural wiring manifests in atypical psychology, in autism.

In the lecture I identify specific non-evolutionary sensory experiences that are inappropriate during development, list a host of supporting evidence, propose prevention and treatment.

SL-103

Track: *Pulmonary Drug Discovery Therapy*

SEX STEROIDS IN ASTHMA

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Clinical evidence suggests differences between males vs. females in the incidence, morbidity and mortality of lung diseases including asthma and COPD. Asthma is more prevalent in pre-pubertal boys than girls, but increases in women with puberty, worsening during pregnancy, and subsiding with menopause. However, unlike cardiovascular diseases, there is currently limited information on inherent structural or functional differences in the airways of males vs. females, or the role of sex steroids (especially female sex steroids) in asthma. The purpose of this talk is to highlight novel bench and clinical research data from our group and other investigators that explain whether and how sex hormone signaling mechanisms could explain clinical observations of male vs. female differences in asthma. The talk will focus on expression and complex signaling of sex steroid receptors in bronchial epithelium (nitric oxide production), airway smooth muscle (calcium regulation and cell proliferation), airway nerves (airway irritability) and inflammatory cells in asthma. These studies will be used to lay the foundation for asking the question: Are sex steroids (especially estrogens) beneficial or detrimental in asthma? Furthermore, the potential use of sex steroid signaling as biomarkers and therapeutic avenues in asthma will be discussed.

SL-111

Track: *Recent Advances in Patient Treatment and Care*

TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS) AS POTENTIAL THERAPEUTIC TOOLS FOR NEUROLOGICAL AND PSYCHIATRIC DISORDERS

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Transcranial direct current stimulation (tDCS) is a noninvasive technique for brain modulation (Priori *et al.* 1998) that induces prolonged functional changes in the cerebral cortex. tDCS delivers a weak (0.5-2 mA) direct current (DC) over the scal: the resulting constant electric field penetrates the skull and influences neuronal function. In general, whereas cathodal tDCS has a suppressive effect, ano dal tDCS increases the function of the underlying areas of the cerebral cortex. Functional neuroimaging studies showed that tDCS elicits long-lasting functional and neurochemical changes in the brain (Lang *et al.* 2005). Currently, tDCS is being studied for the treatment of a number of neurological and psychiatric conditions. tDCS modulates motor function in healthy subjects, in patients after a stroke and with Parkinson's disease. Also, tDCS can improve cognitive performances in healthy subjects and in patients with cognitive impairment (Alzheimer's disease, speech disorders). Furthermore, the technique improves the clinical status in patients with major depression and schizophrenia. Finally, tDCS has been shown to be effective in the treatment of chronic pain. The possibility of modulating the function of the cerebellum and of the spinal cord with DC promises further therapeutic opportunities in the near future. tDCS is a novel non-invasive, inexpensive, simple and valuable potential treatment (adjunctive or alternative to drugs) for psychiatric and neurologic diseases with abnormal brain excitability or function.

Keywords: tDCS, Treatment, New Tool, Depression, Alzheimer, Neurological Disease.

SL-75

Track: *CNS Drug Discovery & Therapy*

SOMATOSTATIN IN MAMMALIAN COCHLEA: IT IS A POTENTIAL THERAPEUTIC WITH PROTECTIVE EFFECT ON COCHLEA?

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Somatostatins (SST) form a family of cyclopeptides that are mainly produced by normal endocrine, gastrointestinal, immune and neuronal cells, as well as by certain tumours. By binding to G-protein-coupled receptors (SST1-5) on target cells, SST act as neuromodulators and neurotransmitters, as well as potent inhibitors of various secretory processes and cell proliferation. The antisecretory profile of somatostatin has led to exploratory clinical trials of natural SST or SST analogues for the treatment of a range of conditions, including: gastrointersinal disease, CNS disease, tumors. Sensorineural hearing loss is one of the most common disabilities in our society today. Little is known about the expression and function of the somatostatinergic system in the mammalian cochlea. First of all we analyzed the expression of SST1, SST2 in the immature mammalian cochlea. SST1 and SST2 immunoreactivity in the auditory sensory epithelium of the developing mouse correlated with other developmental events. In our studies also we detected mRNA expression of all five-somatostatin receptors within the cochlea. The peak in the expression of both receptors at mRNA and protein level is present around the time when they respond to physiological hearing (i.e. hearing to airborne sound), at postnatal (P)14. A similar developmental pattern was observed for SST receptors at mRNA level, suggesting that their gene expression is controlled throughout development at the transcriptional level. Taken together, the dynamic nature of SST receptors expression at a time of major developmental changes in cochlear suggests their involvement in the maturation of the mammalian cochlea. Further in our studies we demonstrated that the all five receptors are expressed in the outer and inner hair cells as well as in defined supporting cells of the organ of Corti in adult mouse cochlea. A similar expression of the SST receptors in the inner and outer hair cells was found in cultivated P6 mouse organ of Corti explants. In order to learn more about the regulation of SST receptors, we used mice with either a deletion of SST1 or SST2. In SST1 knock-out mice, SST2 was up-regulated as compared to wild-type mice. However, in SST2 knock-out mice, the expression pattern of SST1 receptor was not altered compared to wild-type mice. These findings provide evidence of a compensatory regulation in the mammalian cochlea as a consequence of a receptor deletion. In addition, we observed reduced levels of phospho-Akt and total-Akt in SST1 knock- out (KO) and SST1/SST2 KO mice as compared to wild - type mice. We know from previous studies that Akt is involved in hair cell survival. Most importantly, we found improved hair cell survival in somatostatin treated samples that had been exposed to gentamicin demonstrating a protective effect of somatostatin. These findings propose that the somatostatinergic system within the cochlea may have neuroprotective properties.

Keywords: CNS, cochlea, inner ear, somatostatin, somatostatin receptors.

SL-152

Track: *Inflammation & Immunology*

STUDY OF ATORVASTATIN IN EXPERIMENTAL ALLERGIC AIRWAY INFLAMMATION IN MICE

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Background: Inflammation and oxidative stress are associated with airway diseases. There is growing evidence that Atorvastatin could be used as a therapy for these conditions.

Objective: On these bases, we evaluated Atorvastatin as a protective and reversal treatment for the allergic airway diseases in mice model. We also looked at the possible interaction with the currently used effective medication.

Methods: Mice were sensitized and challenged with ovalbumin (OVA) to develop features of allergic airway diseases mainly of bronchial inflammation. Atorvastatin was injected during or after the sensitization and challenge process to

evaluate its protective or reversal effects, respectively. Total and differential cells in the BAL fluids together with IL-4, IL-5 and IL-10 cytokine levels were evaluated. Total IgE and cholesterol levels in serum were studied.

Results: In the protective phase, Atorvastatin inhibited the OVA-induced cellular infiltration of lung bronchi, decreased IL-4 and IL-5 and prevented the increase in IL-10 cytokine levels. Also, it reduced the OVA-induced high serum total IgE level. Injection of Atorvastatin after challenge was not effective in reversing the inflammatory process, with no major contribution towards augmenting the actions of Dexamethasone. The cholesterol lowering effect was marked in the protective phase while less effective for the reversal phase.

Conclusion: Our results indicate that Atorvastatin reduced the allergic inflammatory features in mice and it could be useful towards developing a better therapeutic regimen for the treatment of allergic diseases.

Keywords: Atorvastatin, Bronchoalveolar fluid (BAL), Lung inflammation, Cytokines, Total IgE, Cholesterol.

SL-207

Track: *Cardiovascular Drug Discovery & Therapy*

THE ROLE OF GLYCOPROTEIN 2B/3A INHIBITORS IN ACUTE CORONARY SYNDROMES

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Chronic CHF is common, and of growing importance as a public health problem. Multiple prospective clinical trials document that 30-40 percent of patients with chronic CHF have concomitant diabetes. Despite this high prevalence, there has never been a randomized trial of a specific hypoglycemic therapy in patients with diabetes and CHF. This is important, as recent studies have suggested that some therapeutic approaches to diabetes may have adverse effects in patients with congestive heart failure.Chronic CHF is a complex clinical syndrome with numerous pathophysiologic characteristics that have potentially important interactions with diabetes. These include but are not limited to 1) abnormalities in myocardial energy expenditure and production; 2)dysregulation of the central and peripheral autonomic nervous system; 3) abnormalities in vascular endothelial and smooth muscle cell function and, 4) abnormalities in renal function. It follows that the therapeutic approach to diabetes can have an impact on CHF outcomes and a better understanding of the outcome of diabetic pharmacotherapeutics in chronic heart failure is warranted.A number of animal studies have suggested that agonism of the glucagon like peptide (GLP-1) system has beneficial effects in models of chronic heart failure but human data are limited.Previous animal and human investigations reveal that the GLP-1 system and its pharmacologic manipulation have important interactions with many of the pathophysiologic abnormalities observed in chronic CHF. Given the current interest in the impact of manipulation of the GLP-1 pathway on heart rate and blood pressure, specific investigations of these endpoints in the setting of CHF is warranted and is an important focus of the current study. In this presentation the interaction between CHF and current therapeutic approaches to diabetes will be discussed.

SL-32

ADVANCED APPLICATIONS OF SLIDE-BASED CYTOMETRY IN PHARMACEUTICAL RESEARCH AND CLINICAL STUDIES USING TISSUEFAXS

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Background: Automated identification and cytometric quantification of molecular markers on the single cell level in tissue sections has become an essential tool in pharmaceutical research and clinical studies. TissueFAXS is the microscopic equivalent to flow cytometry – applicable to tissue sections, cell culture monolayers and cell smears/cytospin preparations and allows observer independent analysis *in-situ*.

Technology: The TissueFAXS system consists of a high-end microscope and sophisticated software for image analysis. By using the nuclear marker, cells staining is quantified for each marker. It allows comparison of nuclear/cytoplasmatic marker expression, as dot plots and histograms in a flow cytometry manner.

Results: Results presented come from several studies and comprise different applications of slide-based cytometry.

(i) Phenotypic characterization of tissue infiltrating leukocytes in tumor biology [1], transplantation immunology [2] and autoimmune diseases [3] in renal cell carcinoma, allograft rejection and atopic dermatitis.

(ii) Signal transduction research in prostate cancer [4,5] and brain neuropathology [6]

(iii) Quantification of proliferation marker Ki67 and Her2 in breast cancer.

Discussion: The data above demonstrate novel approaches in pharmaceutical research and improved data transparency in clinical studies using slide-based cytometry. Observer-biased visual estimation in immunohistological and cytological analysis of tissue samples and cell cultures is replaced by observer independent measurements on the single-cell level.

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SL-94

Track: *Hot Topics in Drug Targets*

THE N-TERMINAL RESIDUES OF NS2B COFACTOR REPRESENT THE CORE INTERACTION REGION WITH NS3 THAT ARE CRITICAL FOR ENTIRE ACTIVITY OF DENGUE VIRUS NS3 SERINE PROTEASE

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The NS2b serine protease cofactor of dengue virus is required for entire activity of NS3 protease to cleave viral polyprotein. The region of high binding affinity between NS2b and NS3 that can be used for allosteric inhibition has not fully explored. This study aims to identify the am ino acid residues of NS2b that have crucial role in binding with NS3. In this study, twenty amino acids recombinant peptides derived from NS2b amino acids sequence were produced by *E. coli*. Five amino acids sliding from the N-terminus of 47 amino acids of NS2b produced six peptides (P1 to P6). Using these peptides to interrupt the interaction between NS2b and NS3 and subsequently reduce enzyme activity was used as indicator for peptides binding affinity with NS3 unit. The P1 peptide that represents the sequence from amino acid number 1 to 20 was found to act as binding inhibitor by reducing 67% of enzyme activity (Ki, 172.3 ± 25 μM). The other peptides exhibit less binding inhibition and the reduction in enzyme activity was ranged from 30% to 51% (Ki, range 446.9 ± 66 μM to 896.4 ± 198 μM). Furthermore, the protein pulling down assay using western blot and ELISA indicated that P1 has the highest binding affinity with NS3 unit comparing with other peptides. Data analysis indicated that the residues ADLEL have crucial role in anchoring NS2B with NS3 and the binding affinity reduced toward C-terminal of NS2B. In addition, deletion of these residues abolished enzyme activity. These finding provides important information to develop allosteric inhibitors targeting NS2B-NS3 interaction of dengue protease.

SL-82

Track: *Cardiovascular Drug Discovery & Therapy*

NEW METHOD FOR MONITORING THE EFFECTIVENESS OF DRUGS IMPACT ON CARDIOVASCULAR SYSTEM

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The groundwork for natural sciences is measuring technology. Measuring methods used may be either direct or indirect. It is known that measuring technologies in medicine are very limited in their scope. This is because of lack of the required reference measuring instrumentation. A living body is an integral interrelated system, and it is rather impossible to develop nowadays a theoretical model of any organ that could cover its main features. This is supported by the fact that there are no artificial organs available at present. However, it should be noted that measuring in medicine, including cardiology, is much in demand. In order to provide the required instrumented measuring in cardiology, developed should be a new universal model of hemodynamics to be capable both of describing the blood flow structure and anatomic mechanisms responsible for its maintenance.

The above authors have succeeded in the development of the new blood flow model, the basis of which is the mode of elevated blood fluidity. This flow mode has been simulated in a rigid pipe so that it makes it possible to describe it mathematically. The proposed equations of hemodynamics allow for indirectly measuring blood volumes in every phase of every cardiac cycle. It emerged that it is quite sufficient to use for this purpose one ECG channel only, provided that ECG signals are recorded from the ascending aorta.

Our researches based on phase-related measurements of blood volumes supply us with a lot of radically new data on the function of the heart. Such biophysics interpretation of the heart function makes possible to identify biochemical process levels of the norm and deviations from it, including and up to pathology in the myocardial cells and the septum.

It is evident from the foregoing that it becomes possible now to classify the measured range of the heart function parameters from their norm up to extreme cases of pathology. This is also a pre-requisite to control the efficiency of therapy of different cardiovascular diseases.

Keywords: Cardiology, hemodynamics, cardiovascular system.

SL-202

Track: *Cardiovascular Drug Discovery & Therapy*

PHASE HEMODYNAMIC PARAMETERS MEASURING FOR MONITORING THE TREATMENT EFFECTIVENESS ON CARDIOVASCULAR SYSTEM

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The indirect method of the phase hemodynamic parameters measuring is highly inform ative for monitoring the treatment effectiveness of cardiovascular system. The authors investigated the effect of different pharmacological remedies on the processes of recovery of myocardium contractile function. In the process of research a range of ECG changes from extremely complicated to relatively normal was established. At the same time the reasons influencing the change in shape of ECG were clarified - mitochondrial cardiomyopathy. This pathology is connected with muscle contraction energy reducing. It is spread among population very much and declares itself among the overwhelming number of aged people. It is based on reducing of ATP generation by mitochondria, observed in case of oxygen insufficiency in them.

The report will present the quantitative characteristics of phase volumes of blood, measured by the indirect method, which accurately reflects the reaction of cardiovascular system on drug therapy.

Keywords: Cardiology, hemodynamics, cardiovascular system.

SL-118

Track: *Pharmacogenomics*

PHARMACO-GENOMICS AND THE PROMISE OF INDIVIDUALIZED THERAPY

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Tailoring drug therapy to patients has been an attractive idea for clinical pharmacologists and physicians. The evidence of genetic effect on drug response was first predicted by Sir Archibald Garrot in 1931. More than a decade after the release of "Human Genome" research has been tremendous in this field. The rationale for customized drug therapy is obvious: clinically, most drugs and recommended dosing regimens come with a significant risk of either drug toxicity or treatment failure. In practice, there are three key elements regarding personalized therapies: choosing the drug itself, choosing the dosing regimen and ensuring safety. Despite its apparent simplicity, individualization of treatment is a major challenge due to molecular heterogeneity in of human diseases and marked patient-to-patient as well as between-population differences in drug effects. Major domains that are explored in this field so far focus on the effect of gene polymorphism on drug metabolism, drug transporters and ion channels. Moreover, such research can significantly influence drug discovery: routine application of high-throughput screening (HTS) methods together with combinatorial chemistry and a rich array of chemical libraries greatly accelerated the entry of therapeutic candidates with clearly defined molecular targets to early phase clinical evaluation, but only less than 50% of the drugs approved in the USA between 1990-2000 are truly new molecules. Genome wide association studies of drug response in common diseases may provide clues for discovery of novel drug targets as well as entry points for the development of first-in-class compounds with unique pharmacological mechanism of action and clinically relevant effects. Recent and Milestone researches in pharmacogenomics will be highlighted.

SL-33

Track: *Regenerative Medicine*

OSTEOACTIVIN, A NOVEL PROTEIN FOR STEM CELL DIFFERENTIATION AND BONE REGENERATION

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Osteoactivin (OA) is a novel glycoprotein that is highly expressed during osteoblast differentiation. Subsequent *in situ* hybridization and immunohistochemical localization demonstrated that OA mRNA and protein are expressed by osteoblasts lining the trabeculae in normal long bone. Using Western blot analysis, our data showed that the OA protein has two isoforms, one is transmembranous with a MW of 65 kDa and one is secreted into the conditioned medium of primary osteoblasts cultures and has a MW of 115 kDa. We examined OA protein production in primary osteoblast cultures and found that OA is maximally expressed during the third week of culture (last stage of osteoblast differentiation). The functional role of the secreted OA isoform was revealed that when cultures treated with OA antibody showed decreased osteoblast differentiation compared to untreated control cultures. Gain-of-function of OA in osteoblast using the pBabe viral system showed that OA overexpression in osteoblast stimulated osteoblast differentiation and function. The availability of a naturally occurring mutant of OA with a truncated protein provided further evidence that OA is important factor for osteoblast differentiation and function. Using micro-CT, histomorphometric and histological analyses, mice null for OA have bone loss associated with age. Using bone marrow mesenchymal stem cells derived from OA mutant and wild-type mice and test their ability to differentiate to osteoblasts showed that OA mutant osteoblasts differentiation was significantly reduced compared to normal osteoblasts. Using critical size defect model to test the bone regenerative effects of OA, we found that OA protein stimulates bone regeneration examined by x-ray and histological analyses suggesting that OA stimulate stem cell differentiation to ostoeblasts and bone regeneration. Collectively, our data suggest that OA acts as a positive regulator of osteoblastogenesis in vitro and vivo and OA can be used for potential treatment of osteoporosis and bone loss associated diseases.

SL-165

Track: *Pharmaceutical Research & Development (Global Roundup of Pharmaceutical Research capabilities and Opportunities: Middle East)*

TREATMENT OF HIGHLY FATAL OLEANDER POISONING IN MAN AND ANIMALS IN IRAN, AN OVERREVIEW

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Poisoning with poisonous plants, because of its high rate of mortality has its own significance in veterinary medicine. Oleander (nerium oleander) is one of the highly toxic plants, originally is a diterranean and asian plant and now is widely distributed in the world. It is a very common ornamental tree grown in most parts of IRAN, especially in subtropical areas. All parts of oleander are toxic to animals and human beings, although it is said that the seeds are more toxic than the other parts.

Oleander can induce profound pharmacodynamic effects on the heart. In lower doses cardiac conduction disturbances such as sinus bradycardia, SA block, first and second degree AV block and AV dissociation predominated. It has been reported that digitalis and digitalis-like glycosides have both direct and indirect effects on the cardiac conduction system. Atropin or any other parasympatholytic drugs given at this stage can eliminate conduction disturbances temporarily.

At higher doses, the toxin can either stimulate the sympathetic nerve, producing sinus tachycardia and increase ventricular irritability causing premature ventricular contractions and ventricular tachycardia, or like digitalis inhibit the Na,K-ATPase pump. Thus, the acute toxicity can be manifested by hyperkalemia as reported in oleander poisoning in human beings. Propranolol, lidocaine or similar drugs can act at this stage but with various success. In the case of human poisoning, administration of digoxin Fab antibody fragments has been suggested.

This review deals with the poisoning effects on human and reports poisoning of some cows and calves in dairy herds and evaluate an author experiences on the effect of V285 (vinegar, acetic acid 5%), had told some effects on arrhythmias in previous investigations.

Keywords: Oleander, Poisoning, animal, Treatment, man.

SL-69

Track: *CNS Drug Discovery & Therapy*

GLUTAMATE TRANSPORTER 1: POTENTIAL TARGET FOR THE TREATMENTS OF DRUG ABUSE AND NEURODEGENERATIVE DISEASES.

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Emerging evidence indicates that many aspects of alcohol and drug addiction and neurodegenerative disease involve changes in glutamate transmission. A number of studies have reported that some drugs of abuse, including alcohol and cocaine, also alter glutamate transport. Extracellular glutamate is regulated by a number of glutamate transporters in various brain regions. Of these transporters, GLT1 is a key player in the removal of most of the extracellular glutamate. Similar to disease models including Huntington's disease (HD), in which there is dysfunction of the glutamatergic excitatory system, the role of GLT1 has been tested in drug abuse models that show dysfunction of glutamate transmission. We have recently found that ceftriaxone (CEF), an FDA-approved drug known to elevate GTL1 expression, attenuates cue-induced cocaine relapse. Moreover, we recently found that alcohol-preferring rats treated with CEF showed a significant dose-dependent reduction in alcohol consumption. We also demonstrated that CEF-induced upregulation of GLT1 expression was associated with increases in glutamate uptake and attenuation of HD phenotype in mouse model. Importantly, CEF is currently in clinical trials (phase III) for the treatment of ALS. These

findings suggest that GLT1 is a potential therapeutic target for the treatment of drugs of abuse, including alcohol, and for the prevention of the progression of neurodegenerative diseases including HD.

SL-40

Track: *Innovative Drug Discovery and Nanotechnology*

APPLICATIONS OF CHITOSAN PARTICULATE SYSTEMS IN VACCINE DELIVERY

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Particulate systems offer several advantages as adjuvant/delivery system for vaccines. Pathogens and microparticles are almost in the same size thus immune system reacts in similar way to both of these structures. Subsequently, microparticles can be internalized like pathogens by antigen-presenting cells which results in an action as an adjuvant. Furthermore. particulate delivery systems can present multiple copies of antigens on their surface, which has been shown to be optimal for B cell activation. Chitosan, which is a biodegradable, bioadhesive and biocompatible cationic polymer has been shown to have immune stimulating activity inducing both the humoral and cellular responses. Chitosan based micro- and nanoparticles can easily be prepared which encapsulate larger amounts of antigens without using any organic solvent. They can deliver the antigenic molecules via parenteral and non-parenteral routes. They also provide improved protection and facilitated transport of the antigen as well as more effective antigen recognition by the immune cells which results in enhanced immune responses. Furthermore, versatility in physicochemical properties of chitosan provides an exceptional opportunity to engineer antigen-specific adjuvant/delivery systems. In this presentation, the importance of chitosan in particulate systems for vaccine delivery will be emphasized according to administration routes and recent data of our group will be reviewed.

Keywords: Chitosan, adjuvant, vaccine, particulate systems, needle-free.

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SL-87

Track: *CNS Drug Discovery & Therapy*

DESIGN, SYNTHESIS AND PRE-CLINICAL CHARACTERIZATION OF NOVEL H3 RECEPTOR ANTAGONISTS AS POTENTIAL THERAPY FOR ALZHEIMER'S

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During the last three years, team o2h with the help of funding from DBT has been working on the development of novel H3R antagonists. The O2H 90, O2H91, O2H111, O2H191 and O2H 194 found to be very potent in the binding assay and showed pKi value 8.2 -9.4 (we now have another 10 such compounds identified). These compounds were further tested for the antagonist selection using in-house developed Eu-GTP Functional assay and the compounds were found to be either antagonists or inverse agonists which are useful for the treatment of cognitive disorders like Alzheimer's.

The compounds were screened at 10 micromolar concentrations which is more than 1000 folds higher than the pKi of the compounds at the H3R to ensure the high safety margin and were found to be selective to H3 R against the major CNS GPCRs. The compounds were also tested in-vitro for the drug-likeness using Microxomal stability, CYP inhibition profile (metabolic toxicity), hERG binding assay (cardiac toxicity), Physico-chemical properties, BAMPA assay

(permeability) – indicates high brain penetration, Primary indications are that the compounds will have good bioavailability and we are undertaking the additional studies for PK and tissue distribution.

The in-vivo studies have been carried out on two compounds on mice and the compounds are found to be orally bio-available, have brain penetration abilities and have been also tested at amylgen, France for the in-vivo models of efficacy using the spontaneous alteration and other models of memory.

The recent novelty searches indicate that the compounds are novel and hitherto unreported. As on today, the patent position is strong in our favor and we have filed the Indian final specification in November 2011. This will be followed by the PCT application.

All the data through the design and in-vivo evaluation will be shared during the talk.

SL-92

Track: *CNS Drug Discovery & Therapy*

THE TARGETED DELIVERY OF CNS DRUG ACROSS THE BLOOD BRAIN BARRIER; CHEMICAL MODIFICATION OF DRUG OR DRUG NANOPARTICAL

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The drug development for neurodegenerative disorders are the major challenge to the science in 21st century. Many FDA approved drugs currently available in the market have limitations in crossing the blood brain barrier (BBB) owing to its complicated vasculature posed by the presence of specialized cells. Examples of current research include technologies that are designed to better interact with neural cells, advanced molecular imaging technologies, materials and hybrid molecules used in neural regeneration, neuroprotection, and targeted delivery of drugs and small molecules across the blood–brain barrier.

Batch supercritical anti-solvent precipitation (SAS) process was used to clonazepam (antiepileptic) and mixed PEG 400-CTAB for preparing drug-polymer composite particles. Solutions of Clonazepam and mixed PEG 40O- CTAB in methanol with overall concentrations of 50-150mg/mi and polymer/drug ratios of 1/1-4/1 were sprayed into the CO_2 at 70-200bar and 35-50-C with drug + polymer solution injection rates of 0.85 and 2.5 ml/min. Spherical particles having mean diameters of 1.88-3.97 p-m, distribution ranges of 0.82-9.7 μ m (the narrowest distribution) and 0.91-46,64 μ m (the broadest distribution) were obtained. The drug release-rate of SAS-co precipitated clonazepam drug - mixed PEG 40O- CTAB (1/1) particles was almost 10 times slower than the drug alone. As the ratio of the polymer increased drug release rate also increased due to the wetting effect of mixed PEG 40O- CTAB.

Keywords: – Clonazepam drug, CTAB, PEG-400, SEM, and TEM Images.

SL-159

Track: *Hot Topics in Natural Products*

DISCOVERY OF NEW IXODICIDES: FROM DOCUMENTATION OF ETHNO-VETERINARY PRACTICES TO SCIENTIFIC VALIDATION IN LABORATORY

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The tick is a major problem for cattle producers in subtropical and tropical areas, especially *Rhipicephalus microplus*, and the disease agents transmitted by it, are a constraint to cost-effective production. The most important method of control for this tick is use of synthetic acaricides, but ticks have striking ability to develop resistance against acaricides. Thus, there is an urgent need for new and alternative compounds for tick control. Objective of this study was to document the use of indigenous plants and to evaluate their ixodicidal effect in order to develop new drugs for control of ticks. Documentation of ethnoveterinary medicine was conducted in Semi-hilly areas of Punjab Province, Pakistan. Seventeen

plants, reported by respondents, were selected for evaluation of acaricidal activity and their aqueous-methanol extracts were prepared. *In vitro* trials were conducted in Australia using syringe test. Serial dilutions of plant extracts were prepared in 0.2% solution of TritonX-100 and LC_{99} value in term of % of plant extract was calculated. Out of 17, seven plants were found as potential candidates for development of ixodicidal drug.

SL-110

Track: *Translational Medicine*

PROSTATE CANCER PREVENTION WITH BIOACTIVE FOOD COMPONENTS

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Prostate cancer is a serious public health concern for men in the western countries including United States. Because prostate cancer is often diagnosed in elderly men, a large window exists for intervention to delay onset and/or progression of this disease. Epidemiological studies continue to support the premise that chemicals in our diet may be protective against prostate cancer risk. This association is quite persuasive for cruciferous vegetables (e.g., broccoli, watercress etc.) and allium vegetables (e.g., garlic). This presentation summarizes the results of our recently published and unpublished studies demonstrating prostate cancer preventive effects of bioactive food components, including broccoli constituent sulforaphane (SFN) and garlic constituent diallyl trisulfide (DATS). Using a transgenic mouse model of prostate cancer (TRAMP model), we show that SFN inhibits early stage prostate cancer whereas DATS is effective against late stage poorly differentiated disease. Mechanisms by which these agents prevent prostate cancer are also briefly discussed. These studies were supported by the NCI grants CA115498 and CA113363.

Keywords: Prostate Cancer, Prevention, Sulforaphane, Diallyl Trisulfide.

SL-72

Track: *Anti-Cancer Drug Discovery & Therapy*

FEASIBILITY STUDY FOR BIWEEKLY ADMINISTRATION OF CISPLATIN PLUS VINORELBINE AS ADJUVANT-CHEMOTHERAPY FOR COMPLETELY RESECTED NON-SMALL CELL LUNG CANCER PATIENTS IN A JAPANESE POPULATION

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Background: Adjuvant cisplatine-based chemotherapy has been shown to improve survival in patients with completely resected NSCLC. To evaluate the feasibility and safety of combination of cisplatine (C) and vinorelbine (V) as adjuvant chemotherapy in Japanese patients with completely resected NSCLC.

Methods: Patients with completely resected NSCLC with ECOG PS 0-1 and preserved renal, hepatic, and bone marrow function were enrolled. Simon's optimal two-stage design was applied. Both cisplatin (50 mg/m2) and vinorelbine (25 mg/m2) were given on days 1 and 15, every 28 days. The primary endpoint of this study was the feasibility of this combination in the four cycles of treatment.

Results: Twenty patients were enrolled, and all patients have completed. The patient characteristics are median age: = 61.8 years (range 38-75); gender: 9 females and 11 males; race: all Japanese; all performance status: 0; procedure: 19 lobectomies and 1 pneumonectomy. The stage distribution: 2 stage IB, 1 stage IIA, 7 stage IIB and 10 stage IIIA. Eighteen of 20 patients (90%) have completed 4 cycles, 2 patients; 3 cycles. Ten patients who discontinued the protocol required dose reduction. Treatment was discontinued due to withdrawal from treatment (n=2) : grade 2 elevated creatinine in1, patient refusal in 1 patient, but no treatment-related deaths were observed.

With regard to hematologic toxicity, there were 4 grade 3, 6 grade 4 neutropenias and 3 grade 3 anemias. No grade 3/4 non-hematologic toxicity was observed. The median intensity was 24.4 (range 20 to 25) mg/m2/week with an average of 23.6 (20-25) mg/m2/week cisplatin and 12.5 (range 11.3 to 12.5) mg/m2/week with an average of 12.2 (11.3-12.5) mg/m2/week vinorelbine. The median relative dose intensity of cisplatin was 97.5 (range 72.5 to 100) % with an average of 94.6 (72.5–100) % and that of vinorelbine was 100 (range 80 to 100) % with an average of 97.8 (80–100) %.

Conclusions: Doublet chemotherapy with cisplatine and vinorelbine in the planned doses and schedule was found to be safe and feasible treatment for Japanese patients following surgical resection for NSCLC.

Keyword: Adjuvant chemotherapy, non--small-cell lung cancer.

SL-109

Track: *Innovative Drug Discovery and Nanotechnology*

ADVANTAGES OF USING ANTIBODIES IN ULTRA LOW DOSES AS A NEW PHARMACOLOGICAL APPROACH

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Antibody-based therapy is a rapidly-developing type of effective medicine, but at the moment its use is restricted because of adverse events and high cost. Usage of antibodies in the form of ultra low doses (ULDab) makes it possible to overcome the above-mentioned restrictions and opens new therapeutic potential. We have fifteen years experience accumulated from preclinical and clinical studies of oral ULDab conducted in leading CROs and institutions of EU, USA and Russia, which allows us summarizing the main advantages of this therapy. It has been shown that their efficacy for treating various diseases, including infections (influenza, HIV, herpes), diabetes, obesity, chronic heart failure, etc., is comparable with "gold standard drugs". At the same time ULDab have a favorable safety profile: no drug-related adverse events have been found in 60 mln. patients treated with these drugs. The excellent benefit/risk ratio of ULDab makes them the drugs of choice for usage in combination with other medicines, especially with toxic drugs. The molecular mechanism of ULDab action is not well characterized, however it was shown that ULDab regulate functional activity of their targets, possibly acting as allosteric modulators. Much lower manufacturing cost as compared to therapeutic monoclonal antibodies is also an advantage

Keywords: Antibody, ultra low doses.

SL-28

Track: *CNS Drug Discovery & Therapy*

COMBINED SYSTEMIC AND INTRATHECAL CHEMOTHERAPY FOLLOWED BY HIGH-DOSE CHEMOTHERAPY FOR CNS RELAPSE OF AGGRESSIVE LYMPHOMAS: A CURATIVE APPROACH?

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Background: With a median survival of <6 months CNS relapse of aggressive lymphoma has a dismal prognosis. Standards of therapy have not been defined. A beneficial role of high-dose chemotherapy with autologous stem cell stransplantation (HD-ASCT) has been suggested by retrospective data. In this prospective multicenter phase II study a CNS-directed chemotherapy-only regimen including HD-ASCT was evaluated.

Methods: Immunocompetent patients, 18-65 years old with CNS relapse of aggressive lymphomas received two cycles of high-dose methotrexate 4g/m2 i.v. on day 1, ifosfamide 2g/m2 i.v. on days 3-5 and liposmal cytarabine 50mg i.th. on day 6 and one cycle high-dose cytarabine 3g/m2 i.v. on days 1-2, thiotepa 40mg/m2 iv on day 2 and liposmal cytarabine 50mg ith on day 3. Stem cells were collected after the 2nd or 3rd induction course. Patients without progression received carmustin 400mg/m2 i.v. on day -5, thiotepa 2x5mg/kg i.v. on day -4 to -3 and etoposide 150mg/m2 i.v. on day -5 to -3 followed by ASCT.

Results: Fifteen female and 15 male patients with a median age of 58 (29-65) years were enrolled. Histology was aggressive B-cell non-Hodgkin lymphoma (NHL) in 27 patients and T-NHL in three. Median time to CNS relapse was 8.6 (3-80) months. CNS relapse was intracerebral in 24 and meningeal in 13 (combined in seven) patients; systemic lymphoma was present in six. Pretreatment was CHOP-like in 29 patients, including rituximab in 26. Five patients had initially received a CNS prophylaxis.

Response to induction therapy was 73% (CR=10, PR=12); five patients progressed, two were stable, one was not evaluable for response. Twenty three patients proceeded to HD-ASCT; responses were: CR=15, PR=3, and PD=5. After a median follow up of 13 months the median progression free survival is 12.2 (range 1.5-30.7+) months for all patients and not reached for patients who received HD-ASCT; the median overall survival for the whole cohort is 27.4 (1.7-30.7+) months.

Most frequent Grade ≥3° toxicities were leucopenia (50%), thrombopenia (27%), and infection (27%); and - following HD-ASCT - mucositis (25%) and infection (46%). One patient died due to septic diverticulitis and one developed persisting fecal incontinence 4°.

Conclusions: This is the first prospective evaluation of HD-ASCT in this setting. The protocol is highly effective with tolerable toxicity. A prolonged follow up will determine its curative potential.

SL-205

CARNOSIN AS A LIGAND OF DINITROSYL IRON COMPLEXES

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Polyfunctional dipeptide carnosin (beta-alanyl-L-histidin) is now considered as very perspective pharmacological agent. Long-living cells such as neurons or myocytes contain large amounts of this peptide. Carnosin can react with methylglyoxal (MG) so as with other reactive carbonyl compounds and prevent modification of proteins and other molecules during glycation (non-enzymatic glycosylation) process.

It is also known that dinitrosyl iron complexes (DNIC) which contain nitric oxide (NO) and low molecular weight thiols protect biological molecules against oxidative destruction and account for many physiological NO functions. Because of it we studied formation of carnosin-bound DNIC and influence of MG on this process. It was shown using EPR spectroscopy that DNIC are forming in the system containing carnosin, iron ions and Angeli's salt. The latter is donor of nitroxyl (HNO/NO-) - reduced NO form and is also studied as potential pharmacological remedy. Carnosin interacted with MG but spectra of carnosin DNIC did not change. Methylglyoxal sufficiently stimulated formation of carnosin DNIC but completely suppressed formation of DNIC connected with free histidin. Thus we can make conclusion that iron in carnosin DNIC is coordinated with nitrogen atoms of histidin imidazole cycle. Carnosin DNIC formation can be one of mechanisms for neurons and myocytes adaptation to increasing of reactive carbonyl compounds amount at diabetes hyperglycemia. They can also participate in specific signal and regulatory ways of HNO/NO-. So carnosin DNIC could be the compound which can carry out protective functions at both oxidative and carbonyl stress conditions.

Keywords: Polyfunctional dipeptide carnosin, myocytes, Carnosin, methylglyoxal, glycosylation, EPR spectroscopy.

SL-158

UNTRANSFORMED FUNCTIONAL 3D CELL MODELS OF THE GUT: THE NEW FRONTIER FOR DISCOVERY AND EVALUATION OF ORAL MEDICATION AND NUTRACEUTICALS

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Most of today's preclinical methodologies still rely on decades old routines of animal trials and use of cencerogenic or transformed cell lines in 2D monolayer setups. Since such systems strongly differ from the mimicked host, the human, over 90% of all drugs entering the clinical stage are rejected due to unforeseen side effects or lack of efficiency. New methods are available such as the one utilising untransformed normal cell lines of the gut cultured in a 3D model with secondary co-cultures, such as macrophages, hepatocytes or myocytes, allowing for precise and reliable mechanistic studies developed by our department. These models have been deployed in several recent studies as in the case were we have determined the ability of natural mineral waters to enhance muscle growth linked with clinical evidence. They have been proved to provide accurate, reliable estimation of efficiency and safety thus offering the potential to decrease costs and time consumption in the development of new oral medications and nutraceuticals.

SL-209

Track: *"Anti-Cancer Drug Discovery & Therapy"*

DESIGN, SYNTHESIS, AND ANTICANCER EVALUATION OF QUINOLINE DERIVATIVES

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Quinoline moiety is present in many classes of biologically active compounds. Recently, we have synthesized certain 2-phenylquinoline, 2-furanylquinoline, and 4-anilinofuro[2,3-*b*]quinoline derivatives for anticancer evaluations. Among these compounds tested, 1-[4-(furo[2,3-*b*]quinolin-4-ylamino)phenyl]ethanone (**1**) was the most potent with IC_{50} values of less than 4.2 micromole respectively against the growth of HeLa, SKHep, and CE81T cells and therefore, was further evaluated on its effects of cell cycle distribution. Results indicated lead compound **1** induces cell cycle arrest in G2/M followed by apoptosis. However, some drawbacks such as lack of selective cytotoxicity, poor oral bioavailability, and poor water solubility exhibited by **1** prompted us to search for newer derivatives. Through extensive structural optimization, the new lead **2** which possesses aminoalkoxyimino side chain, exhibited selective anticancer activity, higher oral bioavailability, and higher water solubility than **1**. Xenograghic studies and orthotropic lung cancer model in nude mice indicated that compound **2** exhibited significant efficacy in inhibiting tumor growth *in vivo*. Strategies for further structural optimization will be presented.

SL-34

Track: *CNS Drug Discovery & Therapy*

A NEW TARGET FOR DIAGNOSIS AND TREATMENT OF CNS DISORDERS: AGMATINERGIC SYSTEM

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Agmatine is a polyamine that is produced via decarboxylation of L-arginine by the enzyme arginine decarboxylase. It has been accepted as a novel neurotransmitter in brain. Agmatine exhibited anticonvulsant, antinociceptive, anxiolytic and antidepressant-like actions in animals. In experimental studies, it produces some beneficial effects on cerebral ischemia. Agmatine also interacts with the mechanisms of withdrawal syndromes for several addictive agents. In addition, it modulates some processes of learning and memory. Thus, agmatine may be an important agent for the treatment of central nervous system (CNS) disorders. However, the aberrant release and transmission of agmatine in brain may be associated with mechanisms of several CNS disorders, such as psychosis. Interactions between agmatine and other central neurotransmitter systems, such as the glutamatergic and nitrergic systems, seem to be very important. In light of the current literature, we can expect that the central agmatinergic system may be a new key target in development of novel strategies and approaches for understanding the etiopathogenesis of some important central disorders and their pharmacological treatments. The main objective of this lecture is to investigate the effects of agmatine in CNS and highlight its pharmacological importance in central disorders and drug development.

Keywords: Agmatine; central nervous system (CNS); central disorders

SL-99

Track: *Hot Topics in Natural Products*

A STANDARDIZED FORMULATION OF ORGANIC EXTRA VIRGIN OLIVE OILS (OHO®™) DECREASES LIPID CARDIOVASCULAR RISK FACTORS IN RENAL AND PSORIASIS PATIENTS. oHo INTAKE, ALONG WITH THE TOPIC APPLICATION OF oHo-BASED DERMAL PRODUCTS (oHo-DP), AMELIORATES NOTABLY THE CLINICAL EVOLUTION OF PSORIASIS, ATOPIC DERMATITIS AND GRAFT VS HOST SKIN DISEASE (GVHSD)

Vicente G. Villarrubia, Francisco Borrego-Utiel, José Manuel Gil-Cunquero and Vicente Pérez-Bañasco

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Background. A recent meta-analysis has shown the inability of Mediterranean diets (MDs) to positively modify lipid profile in populations at risk for cardiovascular disease (CVD), although other factors are positively modified, thus partially explaining the benefits of some MD types. Also, it is well known the incapacity of conventional olive oils (OOs) to modify lipid or inflammatory cardiovascular risk factors in patients with chronic kidney disease (CKD). **Rationale.** OOs show different quantitative and qualitative chemistry compositions depending on several genetic and epigenetic influencing factors. oHo consists in a rational combination of at least 3 Spanish extra virgin OOs (Cornicabra, Picual and Arbequina varieties). **Results.** When compared to conventional conventional OOs, oHo exerts the following *in vivo* effects in humans: **A. Lipid profiles.** oHo intake increases significantly HLD-c, and diminish LDL-c, tryclycerides and lipoprotein(a) serum levels in CKD patients and in patients with psoriasis, thereby decreasing significantly their risk of CVD. **B. Inflammatory markers.** oHo intake decreases significantly TNF-α, IL-6 and IL-12 concentrations, and increases IL-10 and IFN-γ serum levels in patients with CKD. **C. Other metabolic markers.** oHo intake increases significantly the levels of insulin-like growth factor binding protein-3 (IGFBP-3), without modifying IGF-1 concentrations. **D. Effects on psoriasis.** 5 out of 6 patients with recalcitrant palmo-plantar psoriasis showed complete response after the combined treatment with oHo plus oHo-DP. 15 out of 15 patients with plaque psoriasis achieved complete response after a 30-days treatment that associated oHo intake plus oHo-DP plus topic fluticasone (half of the recommended dose), and without skin side-effects due to corticoids. **E. Effects on GvHSD.** Two young adult patients who developed GvHSD due to semiallogenic bone-marrow transplantation achieved skin complete response after 15 to 30 days treatment with oHo plus oHo-DP. **Conclusions.** More results will be presented at the Congress. oHo mechanism of action and other questions related to lipid nutrition and immuno-metabolic interactions can be consulted at Villarrubia VG *et al*. Actas Dermosifiologr 2010; 101585-599.

SL-25

Track: *Anti-Cancer Drug Discovery & Therapy*

SUPEROXIDE DISMUTASE 1 AS A TARGET FOR CHEMOSENSITIZATION OF PLATINUM RESISTANT OVARIAN CANCER CELLS

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Platinum-based chemotherapy, such as cisplatin, is the primary treatment for ovarian cancer. However, drug resistance has become a major impediment to the successful treatment of ovarian cancer. To date, the molecular mechanisms of resistance to platinum-based chemotherapy remain unclear. In our previous study using a proteomic approach, more than 90 proteins showed significant expression changes when two pairs of ovarian cancer cell lines, A2780/A2780-CP (cisplatin-sensitive/cisplatin-resistant) and 2008/2008-C13*5.25 (cisplatin-sensitive/cisplatin-resistant), were compared. Bioinformatics analysis suggested several potential pathways that may be involved in platinum resistance. Among these potential pathways, a redox regulated pathway involving superoxide dismutase 1 (SOD1) was targeted in order to further explore its involvement in drug resistance. Inhibition of SOD1 activity in the resistant cells by either small-molecule inhibitors or siRNA enabled partial reversal of platinum resistance. Our data suggest that targeting SOD1 can potentially lead to sensitization of platinum-resistant ovarian cancer cells, and SOD1 may be used as a therapeutic target for chemosensitization of drug-resistant ovarian cancer.

SL-35

Track: *Hot Topics in Natural Products*

LIMONOIDS FROM MANGROVE PLANTS OF THE XYLOCARPUS GENUS AND THEIR BIOACTIVITIES

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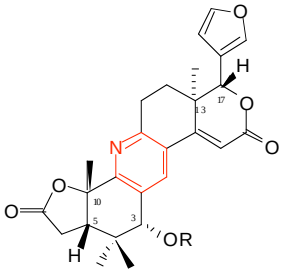
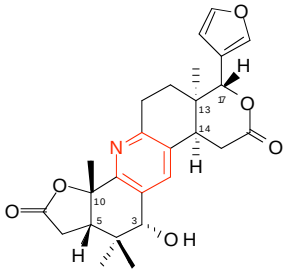
Mangrove plants are a large group of different salt tolerant plants growing in tropical and subtropical intertidal estuarine zones. We will introduce mangrove resources worldwide, and then focus on new limonoids from mangrove plants of the genus *Xylocarpus*. Limonoids, derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton, are tetranortriterpenoids with a beta-furyl ring

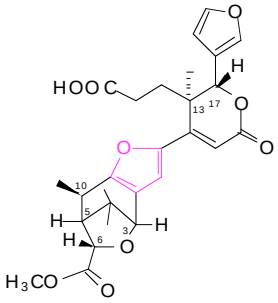
substituent located at C-17. During the recent ten years, my group has identified more than 100 new limonoids from the *Xylocarpus* plants collected in south China and India. The structures of these compounds were elucidated by NMR spectroscopic method combined with single-crystal X-ray diffraction technique. The absolute configurations of limonoids with a new carbon skeleton (Fig. **1**) were established by circular dichroism quantum chemical calculations. Antifeedant, insecticidal, and anti-tumor activities of some limonoids were reported.



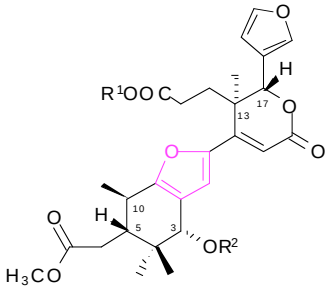
1: R = H (Xylogranatin F)
2: R = $COCH_3$ (Xylogranatin G)



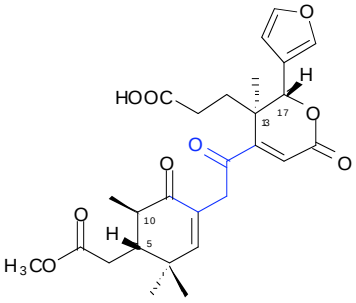
Xylogranatin H (**3**)



12 (xylogranatin Q)



4: R^1 = H R^2 = H (xylogranatin I)
5: R^1 = CH_3 R^2 = CH_3 (xylogranatin J)
6: R^1 = H R^2 = CH_3 (xylogranatin K)
7: R^1 = H R^2 = CH_2CH_3 (xylogranatin L)
8: R^1 = CH_3
R^2 = $COCH_3$ (xylogranatin M)
9: R^1 = H
R^2 = 2*S*-methylbutyryl (xylogranatin N)
10: R^1 = H R^2 = tigloyl (xylogranatin O)
11: R^1 = H
R^2 = isobutyryl (xylogranatin P)



13 (xylogranatin R)

Fig. 1. Structures of Xylogranatins F–R

Keywords: Mangrove; *Xylocarpus*; Limonoids; Bioactivity.

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SL-133

Track: *Hot Topics in Medicinal Chemistry*

NEW DRUG TARGET AND NOVEL CHEMICAL AGENTS FOR MULTIPLE MYELOMA THERAPEUTIC INTERVENTIONS

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Multiple myeloma (MM) remains an incurable malignancy with systematic morbidity and a median survival of 3 to 5 years. A diverse spectrum of agents has shown therapeutic potential in myeloma clinic, e.g., thalidomide, arsenic

trioxide and bortezomib, but high relapse rates and drug resistance continue to plague these therapies. Thus, novel targets and additional pathways need to be discovered to improve the patient outcomes. Here, we report for the first time a discovered novel target for multiple myeloma therapeutic interventions as well as medicinal chemistry synthesis, characterization and biological data related to a novel class of compounds that show promise as candidate therapeutics for treating multiple myeloma. Currently, by using computational and medicinal chemistry, we are exploring structure-activity relationships of various lead compounds to improve the therapeutic potential of this class of molecules.

SL-31

Track: *"Anti-Cancer Drug Discovery & Therapy"*

CORILAGIN IS THE MAJOR ACTIVE COMPOUND FROM *PHYLLANTHUS NIRURI* L. EXTRACTS WITH ANTITUMOR ACTIVITIES IN HEPATOCELLULAR CARCINOMA

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Phyllanthus niruri L. (*P. niruri* L.) belongs to the Euphorbiaceae family and originated in India, which usually occurs as a winter weed throughout the tropic and subtropic parts including China, South Asia, and America. Our garden has introduced and domesticated this plant since 1960's. *P. niruri* L. is a popular folk medicine for treating nephritic, urocystic, gastrointestinal, and hepatic infections. It has been traditionally used for antiviral, antitumor, antioxidant, anti-inflammatory, and antidiabetic treatments as well as for radiation protection. However, all of these medicinal effects were attributed to the crude extracts from *P. niruri* L., the major active compound in the crude extracts remain unknown.

To evaluate the antitumor activity of *P. niruri* L., we isolated components by different chromatographic fractionation methods and identified them by ^{1}H-, ^{13}C-, 2D-NMR and mass spectrometric analyses from the ethyl acetate (EtOAc) fraction of *P. niruri* L. Between two major isolated components (ethyl brevifolincarboxylate and corilagin), corilagin showed better antitumor potential and lower toxicity in normal cells. The IC_{50} of corilagin for normal Chang-liver cells was 131.37 μM, but for hepatocarcinoma (HCC) Bel7402 and SMMC7721 cells were 24.46 μM and 23.36 μM, respectively.

Tumor xenograft model of transplanted MHCC97-H cells, which is a highly metastatic model of HCC, was used to evaluate the antitumor effects *in vivo*. The tumor volume in corilagin treated mice was significantly less than that in negative control mice. Tumor weight in the positive control cyclophosphamide (CTX) treated groups (25 mg/kg) was reduced by 32.93 ± 19.69%, and in the 10 mg/kg, 20 mg/kg, and 30 mg/kg corilagin treated groups were reduced by 17.99 ± 10.56%, 29.88 ± 33.74%, and 47.26 ± 22.65%, respectively, indicating corilagin has same inhibit effects as CTX and with a dose-dependent manner. There was significant difference between negative control and corilagin (20 mg/kg and 30 mg/kg) or CTX groups (P<0.05). More importantly, corilagin treated groups have much less tumor metastasis (only 13.3%), but negative and positive CTX groups had 100% of metastasis, suggesting that corilagin is not only a growth inhibitor, but also a metastasis inhibitor to HCC.

Furthermore, data from flow cytometry and Western blot analyses of cell cycle and cell cycle-related proteins suggest that corilagin arrests SMMC7721 cells in G2/M phase by downregulating p-AKT and cyclin B1/Cdc2 and upregulating p-p53 and $p21^{cip1}$.

In conclusion, corilagin is a major active compound from *P. niruri* L. extracts; it is effective in retarding the growth of HCC cells *via* a p53-$p21^{cip1}$-Cdc2/cyclin B1 signaling pathway. Our finding explored that corilagin could be a novel antitumor drug for HCC treatment.

SL-153

Track: *Pharmaceutical Research & Development (Global Roundup of Pharmaceutical Research capabilities and Opportunities (China))*

PHARMACEUTICAL EVALUATION OF A TRADITIONAL FORMULATION: *ERGH-AL-NESSA* PILL (*HAB*)

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Ergh-al-Nessa pill (*Hab*) is a traditional formulation suggested as one of the most effective preparation used for sciatalgia. Although traditional dosage forms can be applied as new therapeutic drugs for investigations and clinical trials, but they need reformulation to achieve Pharmacopeial standards. In this research, based on seven traditional pharmacopeias (*Qarabadins*), nine different molded tablets was formulated comprised of 0.4g of colchicum, Ginger, Aloe and Myrobalan and 0.3 ml sweet almond oil and needed amount of water.

The pre-formulation studies were performed according to the role of particle size and initial moisture content, on quality control tests such as mass uniformity, LOD (24 and 48 h), hardness, friability and disintegration (20 and 30 min) while the best formulation was in the standard range of 0.24, 4%, 3.1%, 8.31, 0.5%, 3 and 6, respectively, when the particle size of colchicum, Ginger and Aloe was 60-70 mesh size and Myrobalan (mesh size: 30-40) and the initial moisture content was 0.9 ml. The outcome of this research is a pharmaceutical standardized traditional formulation from *Ergh-al-Nessa* pill which can be suggested as a sample of drug discovery based on traditional knowledge.

Keywords: Traditional Pharmacy, *Ergh-al-Nessa*, Sciatalgia.

SL-167

Track: *Enabling Technologies*

SCAFFOLD-BASED DRUG DESIGN: AN EFFICIENT TOOL FOR THE DISCOVERY OF NEW MOLECULAR ENTITIES

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Scaffold-based drug design (SBDD) has emerged over the last decade as an effective drug discovery paradigm that is complementary to conventional high-throughput screening (HTS). The starting point for SBDD is the identification of low-molecular weight and low-affinity scaffold-like compounds that bind to a drug target. These scaffolds can be elaborated to create inhibitors with high affinity and selectivity. Compared with HTS, SBDD requires fewer compounds to be screened and offers more efficient and productive optimization routes despite the lower initial potency of screening hits. I will describe the principles behind the SBDD method. I will use the discovery and development of Zelboraf as an example to illustrate the application and impact of SBDD on the discovery of new drug candidates. Zelboraf is a novel 7-azaindole scaffold containing selective inhibitor targeting the oncogenic mutant of $BRAF^{V600E}$ developed as a strategy to improve the therapeutic index by avoiding the toxic side effects of wild type BRAF inhibition. Zelboraf is a potent inhibitor of $BRAF^{V600E}$ with high selectivity in both biochemical and cellular assays against a large panel of kinases. In melanoma models, Zelboraf induces cell cycle arrest and apoptosis exclusively in $BRAF^{V600E}$-positive cells. The analysis of recently completed phase III clinical trial indicates that metastatic melanoma patients treated with Zelboraf has met the co-primary endpoints on improved overall survival and progression-free survival and has demonstrated clear superiority over the current standard of care. Zelboraf has been approved recently by FDA for the treatment of metastatic melanoma.

SL-139

Track: *Women's Health Drug Discovery and Therapy*

CLINICAL RESEARCH ON GONADOTROPIN RELEASING HORMONE ANALOGUE AND ESTROGEN-PROGESTOGEN ADD-BACK THERAPY IN THE TREATMENT OF ENDOMETRIOSIS

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Objective: To compare the effects of Gonadotropin releasing hormone analogue (GnRHa) alone and GnRHa combined with low-dose dydrogesteronea and estradiol valerate on sex hormone，hypoestrogenic symptoms，quality of life and bone mineral density (BMD) in the treatment of patients with endometriosis.

Methods: Seventy women with moderate and severe endometriosis, who had been diagnosed by surgery, were randomly assigned into two groups as GnRHa group and Add-back group，and treated with Zoladex (3.6mg, i.h.) for three months. Patients in group A took estradiol valerate 0.5mg and dydrogesteronea 5mg every day. Before and after the treatment, assessments were taken, including visual analog scale (VAS), Medical Outcomes Survey Short Form 36 (SF-36), Kupperman Menopausal Index (KMI)，bone mineral density BMD, serum follicle stimulating hormone (FSH), estradiol (E2) and bone gla-protein (BGP).

Results: Sixty-four participants completed the trial, 32 in each group. After the treatment, serum FSH and E_2 in both group declined significantly ($P<0.01$); the level of serum E_2 in Add-back group (93.99±71.05 pmol/L) was higher than that in GnRHa group (53.88±52.08 pmol/L，$P<0.01$); and FSH was lower ($P<0.05$). The value of VAS in both group decreased significantly ($P<0.05$), remaining till menstruated. The score of KMI significantly increased in GnRHa group while the degree of hypoestrogenic symptoms especially hot flashes was minor in Add-back group. Patients in Add-back group had a better life quality, especially in bodily pain and vitality. Bone mineral density of the lumbar spine (L1-L4).

Conclusion: GnRHa and estrogen-progestogen add-back therapy in the treatment of endometriosis is a safe and effective regimen.

SL-208

ATTENTION TO PHILOSOPHY OF SCIENCES AND MULTIDISCIPLINARY RESEARCH CAN IMPROVE PHARMACEUTICAL INDUSTRIES IN THIRD MILLENNIUM

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The third millennium has seen a rise in the number of practitioners of philosophy of a particular science such as philosophy of biological and biomedical sciences, philosophy of chemistry, philosophy of economic, philosophy of mathematics, philosophy of physic, and philosophy of psychology.

Philosophy of science is concerned with the assumptions, foundations, methods and implications of science. It is also concerned with the use and merit of science and sometimes overlaps metaphysics and epistemology by exploring whether scientific results are actually a study of truth. In addition to addressing the general questions regarding science and induction, many philosophers of science are occupied by investigating philosophical or foundational problems in particular sciences.

Multidisciplinary research not only accelerates research progress in areas particularly appropriate to this approach by cross-fertilization of ideas but also help to speed up the conversion of basic research findings to practical application.

This lecture will describe about Metaparadigm, Domain, Philosophy, Phenomenon, Concept, Construct, Proposition, Model, Theory, Paradigm, Discipline, Research, Sciences, multidisciplinary research, and knowledge development based on integration of these items regarding philosophy of sciences. Then, manager of pharmaceutical industries will understand that how attention to philosophy of sciences and multidisciplinary research can improve pharmaceutical industries in third millennium?

POSTERS

PO-61

Track: *Pharmaceutical Research & Development*

OPTIMIZATION AND *IN VITRO-IN VIVO* RELATIONSHIP OF CONTROLLED-RELEASE MICROPARTICLES LOADED WITH TRAMADOL HYDROCHLORIDE

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Development and optimization of controlled release microparticles of tramadol hydrochloride (TmH) for the oral delivery and their *in vitro* and *in vivo* correlation was prime objective of the present study. Four formulations of controlled-released microparticles were developed and optimized in terms of encapsulation efficiency, dissolution study and release kinetics. Among all formulated microparticles F-3 (ratio of drug:polymer 1:2) and F-4 (ratio of drug:polymer 1:3) presented the better characteristics in reverence to entrapment efficiency, release kinetics and dissolution profile compared to other formulations (F-1, F-2). For *in vivo* analysis of drug in body fluid, a new HPLC analytical method was developed and validated. The optimized formulations were subjected to *in vivo* studies to calculated various pharmacokinetic parameters i.e., Cmax, tmax, AUC 0-∞ and MRT. The *in vitro* dissolution and *in vivo* absorption data was corrolated with the help of Wagner-Nelson method. F-3 showed a good *in vitro-in vivo* correlation with a correlation determination of 0.9957. Moreover, lower Cmax and higher values of Tmax and MRT were observed for F-3. The control formulation (immediate-release) presented lowest values of t1/2, MRT and Tmax but the highest values of Cmax and Ke. In conclusion, the controlled-release microparticles of TmH can be developed via phase separation method. The controlled release microparticles could sustain the drug delivery within therapeutic level up to 24 hours and good IVIVC is expected from them.

Keywords: *Vitro-In Vivo* Relationship, Controlled-Release Microparticles.

PO-74

Track: *Biologics*

EFFECTS OF CULINARY-MEDICINAL MUSHROOMS EXTRACT ON MACROPHAGE ACTIVATION *IN VITRO*

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A mushroom characteristically contains many different bioactive compounds with diverse biological activities. The bioactivities of these compounds depend on how the mushroom is prepared and consumed. Hence in this study, mushrooms were boiled in water for 30 minsto simulate cooking condition and the water extract was evaluated for macrophage activation. Polysaccharides in particular β-glucans, have been reported to stimulate the activity of various immune cells like macrophages, T-cells and B cells. The level of β-glucanin hot water extracts of mushrooms ranged from 1. 09 to 17. 92 %. The genus *Pleurotus* are the richest source of β-glucans while *Hericeumerinaceus, Termitomycesheimii* and *Agaricusbisporus* have the lowest value. Macrophages play an important role in immunosurveillance against malignant cells and pathogen. Phagocytosis by macrophages can reflect the individual immune response and can be used as an important marker to improve the disease resistance. *In vitro* uptake of neutral red by RAW264. 7 mouse macrophage cell line indicated that 14 species showed high stimulation of phagocytosis compared to untreated cells except *P. citrinopileatus, P. sajor-caju* and *Schizophyllum commune. Pleurotus florida* and yeast beta-glucan showed highest stimulation of phagocytosis at the lowest concentration of 25 ug/ml. Interestingly, hot water extract of *L. edodes* fruiting bodies showed comparable phagocytosis to the polysaccharide lentinan. The proliferation of macrophage cells was not concentration dependent and *Pleurotus flabellatus* exhibited highest proliferation followed by *P. eryngii*. Further work is in progress to confirm the effect by measuring NO production a mediator of macrophages that acts as a destroyer to bacteria and tumour cells. This evidence extends that regular consumption of mushrooms in human diets is beneficial in immune system enhancement and may have chemopreventive properties of selected diseases of humankind.

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PO-145

Track: *Natural Products*

SIMULTANEOUS IDENTIFICATION OF PHENOLIC COMPOUNDS AND ANTIBACTERIAL ACTIVITY OF THE RAISIN EXTRACT

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In this study, total phenolic compounds from raisin were extracted and their antibacterial activities were studied. Different solvent extraction methods were used to get phenolic compounds from raisins that were investigated using DPPH assay and HPLC technique. The phenolic compounds identified in the dried raisin were catechin, quercetin and rutin. The antibacterial activity of the acetone extract was then tested against four bacterial pathogens viz. *Staphylococcus aureus, Pseudomonas aeroginosa, Escherichia coli* and *Salmonella spp.* using disk diffusion and minimum inhibitory concentration (MIC) method. The extract of raisin inhibited the growth of all the tested bacteria. The results obtained by disc diffusion showed that Staphylococcus aureus were more sensitive to acetone extract whereas no significant activity of the extract was seen against *E. coli* and Salmonella spp.

Keywords: Raisin, phenolic content, antibacterial, antioxidant activities.

PO-75

Track: *Biologics*

ANTIBACTERIAL ACTIVITY OF CLOVE, CINNAMON, AND DATURA EXTRACTS AGAINST ERWINIA CAROTOVORA SUBSP. ATROSEPTICA CAUSATIVE AGENT OF BLACK STEM AND SOFT ROT ON POTATO

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This study was carried out to evaluate the antibacterial activity of Clove (Eugenia caryophylata) hexane extract, Cinnamon (Cinnamomum zelanicum), and Datura (Datura metel) ethanol extracts against the phytopathogenic Erwinia carotovora subsp atroseptica growth, causative agent of black stem and soft rot on potato, in both of culture media and on plants in pots under natural conditions. Concentrations of 0.2, 0.5, 1, 3, 5% of each extract were subjected for preliminary antibacterial assays against E.c. atroseptica in nutrient agar by pour plate method. The results of *in vitro* antibacterial activity showed that hexane extract (essential oil) of Clove at 0.2% exhibit the highest inhibitory effect against the pathogenic bacteria, 100% compared to 80% and 90% with ethanol extracts of Cinnamon and Datura at the same concentration. The complete inhibition of E.c. atroseptica growth by cinnamon and datura extracts was achieved at 0.5% concentration. Similar results of the antibacterial activity of the extracts on E.c. atroseptica, on the plant in the pots under natural conditions, were obtained. The greatest suppression of potato black stem and soft rot disease was obtained on plants grown from extract-treated tubers sown in bacteria contaminated soil as well as on plants grown from bacteria-contaminated tubers sown in extract-treated soil (zero infection), compared with untreated tubers in contaminated soil, or contaminated tuber in untreated soil. The application of extracts on potato plant foliage grown from bacteria-contaminated tubers or in bacteria-contaminated soil had no effect on disease incidence, but the application of the extracts on the foliage before bacterial contamination was highly inhibited the disease incidence.

Keywords: Clove, Cinnamon, Datura, plant extracts, Erwinia carotovora subsp. Atroseptica.

PO-127

SYNERGISM FROM COMBINATIONS OF *CIS*- AND *TRANS*-PLATINUMS IN THE HUMAN OVARIAN TUMOUR MODELS

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Cisplatin and its analogue carboplatin are two of the most widely used drugs in the treatment of various cancers including the ovarian cancer. With the aim of reducing the side-effects and widening the spectrum of activity, thousands of cisplatin analogues have been prepared by changing the nature of the leaving groups and the carrier ligands. Although it has been possible to greatly reduce the side-effects, only a limited change in spectrum of activity has been achieved, because of inherent or acquired resistance. Combination of drugs with different mechanisms of action can offer a means of overcoming drug resistance and reducing the side effects provided the drugs act synergistically in combination. Although the antitumour activity of platinum drugs are believed to be associated with their binding with the DNA, the actual nature of interaction of the rule-breaker platinums such as platinum compounds with trans-geometry and multiple metal centres may differ from that of cisplatin analogues. Cisplatin forms mainly intrastrand 1,2-bifunctional Pt(GG) and Pt(AG) adducts whereas *trans*-platinums are expected to form mainly interstrand 1,2-Pt(GG) adduct and multicentred platinums are expected to form long-range inter- and intra-strand Pt(GG) adducts. Hence the rule-breaker platinums may act synergistically in combination with cisplatin. In this study, synergism from combinations of five *trans*-planaramineplatinum(II) complexes, coded as YH11 and YH12 of the form *trans*-$PtL(NH_3)Cl_2$, where L=3-hydroxypyridine and imidazo(1,2-a)pyridine, CH1, CH2 and DH3, of the form *trans*-$PtCl_2L_2$, where L=3-hydroxypyridine, 4-hydroxypyridine, 2-hydroxypyridine in human ovarian tumour models are investigated using three different sequences of administration 0/0 h, 0/4 h and 4/0 h. The sequences 0/0 h, 0/4 h and 4/0 h mean respectively that both the drugs were added at the same time, cisplatin was administered first followed by *trans*-platinum 4 h later and 4/0 h indicate the converse. For combination study cell viability is quantified by MTT reduction assay and results are analyzed by Calcusyn software to provide dose response curves and combination indices (CIs) as measures of synergism, addictiveness and antagonism. This presentation will give an account of the combination studies.

PO-79

SYNTHESIS OF 4-METHYLENE-1,3-NAPHTHOXAZINES BY THE REACTION OF IMINES WITH TRIPHOSGENE

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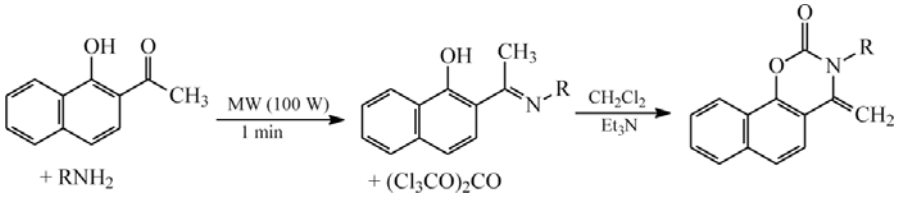
Triphosgene has been repeatedly used in the literature for the construction of a variety of heterocyclic systems. Examples of important heterocyclic systems prepared using this reagent include benzothiadiazepines [1], diazolidines, imidiazolidines and azetidines [2]. We have recently reported the use of triphosgene in the cyclization of hydrazones of 2-acetyl-1-naphthol and 1-acetyl-2-naphthol to give naphthoxazine and spiro naphthoxazine [3]. We report results obtained from the cyclization of imines of 2-acetyl-1-naphthol with triphosgene.



Keywords: 2-Acetyl-1-naphthol; amines; imines; microwave irradiation; 1,3-naphthoxazines; triphosgene.

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PO-155

CARDIA HAEMANGIOMA- TO OPERATE OR NOT TO OPERATE

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Cardiac haemangiomas are rare forms of cardiac tumours whichcan present at any age and in any cardiac location. The clinicalpresentation of patients differs with various presenting symptoms such as dyspnoea,palpitations, atypical chest pain and arrhythmiashaving been reported. Clinical presentation also depends on the location of the tumour and its evolution. Surgical resection is often the treatment of choice. Here, we report a particular case of cardiac haemangioma which was referred to us and eventually managed conservatively.The surgical risks and frequencyof debilitating symptoms associated with this particular haemangioma weretaken into consideration and contrary to common management,surgical excision of the tumour was not performed. The medical history, physical examination, haemangioma evolution as well as our clinical findings at our 1 year follow up are further discussed. We also discuss another case of cardiac haemangioma which required a surgical intervention. The purpose of this review is to discuss the various surgical and non-surgical options available, elicit the various factors that should be taken into consideration when choosing a conservative/surgical approach and stress the need to weigh in the surgical risks and frequencyof debilitating symptoms before excluding a conservative management therapy.

PO-126

MAXIMISING THE CELL KILL DUE TO PLATINUM DRUGS IN THE HUMAN OVARIAN TUMOUR MODELS

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Although cisplatin and its progeny carboplatin and oxaliplatin are routinely used in the clinic to kill cancerous cells, the use of the drugs has also been limited due to intrinsic and acquired resistance. Thus currently intense research effort is being applied with the aim of achieving means to overcome the drug resistance. The multi-factorial nature of platinum resistance means that many different strategies may be required or gainfully employed to overcome the mechanisms of resistance. These may include ways of increasing platinum accumulation within the cell, lowering of deactivation of platinum drug that constantly takes place before its binding with the DNA, increasing the level of platinum-DNA binding and reducing tolerance of platinum-DNA adducts. Cisplatin is believed to cross the cell membrane by carrier-mediated transport in addition to passive diffusion and pinocytosis. One carrier involved in the transport of cisplatin into the cell is the copper transporter CTR1. However, cisplatin is found to trigger the down-regulation of the carrier and its proteasomal degradation. Bortezomib, a proteasome inhibitor, has been reported to block cisplatin-induced down-regulation of CTR1 so that in the presence of the inhibitor cellular uptake of cisplatin and hence the level of its binding with the DNA may be increased. In this study synergism in activity from the sequenced combination of cisplatin and bortezomib in the human ovarian A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$ cancer cell lines has been investigated. Addition of bortezomib 2 h before that of cisplatin is found to produce a greater cell kill than the converse and the bolus, especially in the resistant $A2780^{cisR}$ and $A2780^{ZD0473R}$ cell lines, in line with the increased platinum accumulation and the platinumDNA binding level. Thus the prevention of CTR1 degradation by bortezomib may be playing a significant role in increasing the cellular uptake of cisplatin, platinum-DNA binding level and eventually in the cell kill, especially in the resistant cell lines. We also investigated the effect on the cell kill due to the administration of cisplatin in two aliquots with a time gap. Since cisplatin brings about apoptosis by multiple pathways one of which may be more be dominant than another depending on the status of the cell, it is thought that administration of cisplatin in two aliquots with a time gap may amount to the sequenced combination of two drugs with somewhat independent mechanisms of action and hence may produce synergistic outcomes. Indeed the administration of cisplatin in two aliquots with a time gap is found to maximise the cell kill in the human ovarian cancer cell lines. If found to be true *in vivo*, the above results may have a profound clinical significance.

Keywords: Cisplatin, ovarian cancer, bortezomib, drug resistance, MTT, sequenced combination.

PO-55

Track: *Pharmaceutical Research & Development*

A VALIDATED CAPILLARY ELECTROPHORESIS METHOD FOR SIMULTANEOUS DETERMINATION OF EZETIMIBE AND ATORVASTATIN IN PHARMACEUTICAL FORMULATIONS

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Ezetimibe and atorvastatin are used for treating hypercholesterolemia. Clinical studies have shown that co-administration of ezetimibe plus atorvastatin was significantly more effective at reducing cholesterol and triglycerides than ezetimibe or atorvastatin alone, and the clinical use of these two agents as a combination is increasing continuously. Therefore, a simple, precise, and sensitive capillary electrophoresis technique coupled with a diode array detector has been developed for the separation and simultaneous determination of ezetimibe and atorvastatin in pharmaceutical formulations. Separation of both ezetimibe and atorvastatin was achieved utilizing fused silica capillary (58 cm x 75 µm ID) and background electrolyte solution that consisted of phosphate buffer (2.5 mM, pH 6.7): methanol (70:30 v/v). The proposed method was validated by testing its specificity, linearity, precision, accuracy, recovery, and detection limit/quantitation limit values. The method was linear over the range 2.5-50 µg/ml for ezetimibe ($r = 0.9992$) and 1-100 µg/ml for atorvastatin ($r = 0.9999$). Within-day and between-day RSD for ezetimibe and atorvastatin were $\leq 5.6\%$ and $\leq 2.9\%$, respectively. The detection limit was 0.07µg/ml for ezetimibe and 0.06µg/ml for atorvastatin. The validated method was successfully employed for the determination of ezetimibe and atorvastatin in tablets with no interfering peaks from common pharmaceutical excipients. The percentage recoveries of the two drugs from their tablets were 99.80±1.76 and 100.19±1.83 respectively.

Keywords: Ezetimibe, Atorvastatin, Capillary electrophoresis, Diode array detector, Tablets..

PO-32

Track: *Hot Topics in Medicinal Chemistry*

EVALUATION OF ANTI-DIABETIC ACTIVITY AND TOXIC POTENTIAL OF LYCIUM SHAWII IN ANIMAL MODELS

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The powder and decoction of *Lycium shawii* Roem and Schult (Solonaceae) aerial part are used as a folklore remedy in the treatment of diabetes by the local community in various parts of Saudi Arabia. In the present study, attempts were made to scientifically justify the alleged anti-diabetic efficacy of this plant and to evaluate its toxic potential. The 80% ethanol extract of *L. shawii* aerial parts was prepared.

After evaporation of ethanol, it was freeze dried. A statistically significant blood glucose lowering effect was noticed in Long-Evans rats treated orally with 250 mg/kg ($P<0.05$) and 500 mg/kg body weight ($P<0.001$) of *L. shawii* extract. In addition, there was a significant decrease in blood glucose levels of animals treated with the extract with a simultaneous load of glucose (2.5 mg/kg). A significant ($P<0.001$) anti-diabetic effect was also observed in streptozotocin (STZ) diabetic rats. The data obtained clearly justified the claimed hypoglycemic activity of *L. shawii*. To demonstrate any toxic potential of *L. shawii* treatment, acute (24 h) and chronic (90 days) toxicity studies were conducted using mice as experimental model. Acute dosages were 0.5, 1.0 and 3 g/kg body weight (gavage) while chronic dosage was 100 mg/kg per day of the extract in drinking water. All morphological, biochemical, haematological and spermatogenic changes, in addition to mortality, body weight changes and any change in vital organs were recorded and compared with the respective control groups. Histopathological investigations were done on vital organs and compared with the control mice without treatment. *L. shawii* chronic treatment induced changes in body weight, biochemical and hematological parameters and was found to possess significant spermatatoxic potential.

Keywords: *Lycium shawii*, blood glucose lowering potential, streptozotocin diabetes, toxicity biochemical and hematological effects.

PO-62

Track: *Pharmaceutical Research & Development*

A VALIDATED REVERSE PHASE LC METHOD FOR THE SIMULTANEOUS ESTIMATION OF FEXOFANADINE HCL AND PSEUDOEPHEDRINE HCL IN PHARMACEUTICAL DOSAGE FORM USING A MONOLITHIC SILICA COLUMN

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A simple, rapid, accurate, precise and sensitive isocratic reverse phase high performance liquid chromatographic method (RP-LC) has been developed for the simultaneous estimation of Fexofenadine HCl (FEX) and Pseudoephedrine HCl (PSE) in pharmaceutical dosage forms from their combination formulation. The proposed method utilized Chromolith® Performance, RP-18e , 100 × 4.6mm column and the separation was achieved with mobile phase consisted of phosphate buffer pH 4.5: acetonitrile : methanol (65: 25 : 10 v/v) , delivered at a flow rate of 2.4 ml/min and wavelength of detection at 258nm. Losartan was chosen as the internal standard which elution is between the two tested active ingredients to guarantee a high level of quantitative performance. The method has shown adequate separation using Losartan with good resolution to both of the active ingredients, in which contest the retention time of Pseudoephedrine HCl, Losartan and Fexofenadine HCl were 0.7, 2.1 and 2.7 min, respectively. The described method was linear over the range of 0.8- 100 µg mL-1 (r=0.9995) for Fexofenadine HCl and 2.0 - 200 µg mL-1 (r=0.9999) for Pseudoephedrine HCl. Intra- and inter-day % RSD (n=6) was ≤ 1.0%. The percentage recovery of the two drugs from their tablet formulation were 99.5 ± 0.3 (FEX) and 99.6 ± 0.4 (PSE). The developed method was validated according to ICH guidelines .The method can be used for rapid and accurate quantitative determination of these drugs in combined dosage forms.

Keywords: Fexofenadine hydrochloride, Pseudoephedrine hydrochloride, RP-LC, monolithic column, tablets.

PO-91

Track: *Cardiovascular Drug Discovery & Therapy*

PROTECTIVE EFFECTS OF VITAMIN E AND SELENIUM AGAINST DIMETHOATE INDUCED CARDIOTOXICITY *IN VIVO*: BIOCHEMICAL AND HISTOLOGICAL STUDIES

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There is a considerable interest in the study of free radical-mediated damage to biological systems due to pesticide exposure. However, there is a lack of consensus as to which determinations are best used to quantify future risks arising from xenobiotic exposure and natural antioxidant interventions. Our study investigated the potential ability of selenium and/or vitamin E, used as nutritional supplements, to alleviate cardiotoxicity induced by dimethoate. Female Wistar rats were exposed for 30 days either to dimethoate (0.2 g/L of drinking water), dimethoate+selenium (0.5 mg/kg of diet), dimethoate+vitamin E (100 mg/kg of diet), or dimethoate+selenium+vitamin E. Exposure to dimethoate increased malondialdehyde, advanced protein oxidation and protein carbonyl levels, glutathione peroxidase, superoxide dismutase and catalase activities in the heart tissue. A decrease in acetylcholinesterase and Na^+K^+-ATPase activities, glutathione, non-protein thiols, vitamins C and E levels was observed. Plasma levels of cholesterol, triglycerides and low density lipoprotein-cholesterol increased, those of high density lipoprotein-cholesterol decreased. Co-administration of selenium or vitamin E to the diet of dimethoate treated rats ameliorated the biochemical parameters cited above. But the joint effect of selenium and vitamin E was more powerful in antagonizing DM-induced oxidative stress. The histopathological findings confirmed the biochemical results and the potential protective effects of selenium and vitamin E against cardiotoxicity induced by dimethoate.

Keywords: Dimethoate, Selenium, Vitamin E, Rats, Cardiotoxicity, Antioxidant and lipid profiles, Histopathological studies.

PO-71

EVALUATION OF EFFICACY PERSIAN SHALLOT (*ALLIUM HIRTIFOLIUM*, BOISS) AQUEOUS EXTRACT ON MOUTH BACTERIAL COUNT COMPARED WITH CHLORHEXIDINE MOUTH RINSE

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Background: This study attempted to compare Persian shallot aqueous extract with commercially available chlorhexidine (CHX) mouth rinse with respect to their anti-microbial activity on salivary bacterial counts.

Methods: Three groups of 10 volunteers, with a healthy oral status, were randomly enrolled. Using sterile water as negative control, standard test group using 0.2 % CHX as standard control and Persian shallot aqueous extract for test group. A single mouth rinse lasting 15 s of each mouth rinse was employed. Salivary bacterial counts were obtained by collecting unstimulated saliva samples at the beginning before rinsing for measurement of baseline count and 1, 5 and 24 h after rinsing with the assigned solution. Analysis of variance and Bonferroni *post hoc* tests were used to evaluate significant differences among groups.

Results: No significant differences among the allocated groups were detected at baseline. CHX produced more significant reduction of salivary bacterial count relative to both shallot and distilled water control at 1 h. In addition a significant difference at 1 h was also detected between both CHX and shallot extract with distilled water. Furthermore, at 5 h there was a significant difference in bacterial count between CHX and shallot extract. After 24 h, the level of bacterial count in shallot extract was still significantly lower than both CHX and distilled water control groups.

Conclusions: The results of this study suggest that Persian shallot extract has more persistent inhibitory action than CHX mouth rinse lasting up to 24 h.

PO-72

IN VITRO COMPARISON OF THE EFFECT OF GARLIC EXTRACT AND CHLORHEXIDINE MOUTHWASH ON ORAL PATHOGENS

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Background: Garlic is a strong antimicrobial agent and acts as an inhibitor on both gram-positive and gram-negative bacteria. The present study was conducted to compare the effect garlic extract and chlorhexidine mouthwash on oral pathogens.

Methods: Fresh garlic bulb were used to separate the antibacterial extract. The bacteria that were tested: *Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius and Lactobacillus casei*. After cultivating the bacteria, minimal inhibitory concentration(MIC) of garlic extract and chlorhexidine were measured by E-test method, then minimal bactericidal concentration(MBC) of chlorhexidine and garlic extract were measured by tube test.

Results: The least MIC of garlic extract for *Streptococcus mutans*, was 0.25 µg/ml and the most was for *Lactobacillus casei* 2.5 µg/ml. The MIC of chlorhexidine for these tow bacterial were 0.62 and 5 µg/ml respectively. The MBC of chlorhexidine and garlic for *Streptococcus mutans* was least concentration than that for other tested bacteria. The MBC of chlorhexidine and garlic for *Streptococcus mutans* was 0.35 and 0.3 µg/ml respectively. The highest MBC of chlorhexidine was for *Streptococcus salivarius* (10 µg/ml). The MBC of garlic for *Streptococcus sanguis* also was 10.4 µg/ml.

Conclusions: The efficacy garlic extract was more than chlorhexidine against target bacteria and can be used as a new mouthwash but its side effects must be investigated.

Keyword: Garlic extract, oral pathogens, mouthwash.

PO-76

Track: *Biologics*

ANTI-ANGIOTENSIN CONVERTING ENZYME (ACE) PROTEINS FROM THE MYCELIA OF *GANODERMA LUCIDUM*

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Ganoderma lucidum has been purported as a potent remedy in the treatment and prevention of several ailments including hypertension. This study aimed to explore the anti-ACE potential of protein fractions from the mycelia of *G. lucidum*. Preliminary result demonstrated that the mycelia crude water extract inhibited ACE at IC_{50} value of 1.134mg/ml. Following protein fractionation and HPLC purification; the presence of highly potential anti-ACE proteins with the IC_{50} values less than 0.02mg/ml was detected. Characterization of these proteins using proteomics platform demonstrated the presence of four different proteins which are involve in the regulation of blood pressure via different mechanisms. This study suggests that the mycelia of *G. lucidum* has high potential in lowering blood pressure level due to the presence of several anti-hypertensive related proteins such as cystathionine beta synthase-like protein, DEAD/DEAH box helicase-like protein, paxillin-like protein and alpha beta hydrolase-like protein.

PO-15

IN SEARCH OF NEW TUMOUR ACTIVE PLATINUM DRUGS STRUCTURALLY AND FUNCTIONALLY DIFFERENT THAN CISPLATIN

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Although used in the clinic, the use of cisplatin has also been limited due to the presence of side effects and development of drug resistance [1, 2].Currently much research efforts are being applied in designing new platinum compounds that would violate classical structure-activity requirements of cisplatin in one way or another. The target compounds of the present study can form monofunctional adduct with DNA, can undergo stacking interaction with nucleobases in the DNA and also take part in H-bonding. This project aims to design new planaramineplatinum(II) compounds of the form $[PtL_3Cl]Cl$ where L= planaramine and to investigate the compounds for antitumour activity, nature of binding with DNA and mode of transport across the cell membrane. The compounds would bind with DNA somewhat differently than cisplatin so that they may have altered spectra of activity as the repair enzyme may not recognize the adduct formed. Because of the charged nature of the compounds, they are expected to cross the cell membrane only by carrier-mediated transport. The compounds can be excellent substrates for organic cation transporters (OCT) 1 and 2, also designated as SLC22A1 and SLC22A2 respectively. Elemental analyses and spectral studies are employed to characterize the compounds whereas MTT reduction assay is utilized to determine activity of the compounds against ovarian cancer cell lines. Results thus far show that the reduction inactivity in going from the parent to the resistant cell lines is less pronounced for the designed complexes than cisplatin so that they have lower resistance factors. Combinations of designed complexes with cisplatin are applied to ovarian tumour models using different sequences of administration with the aim of overcoming drug resistance. Cellular accumulation of platinum and level of Pt-binding are determined to explore structure-activity relationships.

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PO-150

Track: *Structural Biology*

THE *"CLINICAL INTERACTIVE MULTIMEDIAL ALGORHYTHM PROJECT FOR UNIPOLAR DEPRESSION (CIMAP-UD)"*. A TOOL FOR RATIONAL TREATMENT DECISION AND EVALUATION OF EFFECTIVENESS OF PHARMACOTHERAPY IN CLINICAL DEVELOPMENT AND PRACTICE

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The evaluation of the overall effect of antidepressant drugs is crucial in the treatment of Depressive Disorders and especially of Major Depressive Episodes.

Several clinical variables and modes of treatment response, both respect to therapeutic action and side-effects, play a role in influencing the real effectiveness of treatments. Many clinical decisions are requested along a treatment period, both in clinical practice and in preclinical trials on developing drugs.

The currently available algorhythms are structured on fixed possibilities of treatment choices, are poorly sensitive to single patients' clinical peculiarities and don't provide sufficient information about the rational basis of the decisional tree.

The *Clinical Interactive Multimedial Algorhythm for Unipolar Depression* (CIMAP-UD) is a new clinical and research instrument, completely based on interactive and multimedial modalities.

Information on patient's clinical profile (psychopathology, biorhythms, cognition) are compared to both neurobiological models of depression and drugs mechanism of action and clinical indications, presented in single windows of the system.

The therapeutic response is evaluated according to both symptom remission and tolerability and a decisional flow is automatically generated for each treatment according to all the considered variables, including possibilities of treatment maintenance, augmentation or change.

The system can dialogue on patient's individual therapeutic need and is constantly up-dated, also based on progressively stored data, aiming at achieving the excellence level of therapeutic conduct and outcome.

PO-37

Track: *Hot Topics in Natural Products*

RADICAL SCAVENGING ACTIVITY OF POLYFLORAL HONEY WITH DRIED PLUMS

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Honey is a good source of natural antioxidants, which are effective in reducing the risk of heart disease, cancer, cataracts, immune-system decline, different inflammatory processes etc. Dried plums contain higher levels of polyphenols and show stronger antioxidant activity than most other fruits. The aim of this research was to investigate the contribution of addition (20-40% mass) of dried plums to phenoli c and flavonoid contents and radical scavenging activity (RSA) of Homolje honey - Serbian polyfloral honey with appellation of origin. Total phenolic (TP) and flavonoid contents (TF) were determined spectrophotometricaly. RSA on superoxide anion ($O_2^{\cdot-}$) and hydroxyl radicals (.OH) were investigated by electron spin resonance spectroscopy. TP and TF in Homolje honey (19.78mg/100g and 2mg/100g, respectively) was increased after addition of dried plums in a dose dependent manner. Highest TP (46.69mg/100g) and TF (32.59mg/100g) were determined in honey sample with 40% of dried plums. RSA of Homolje honey ($EC50O_2^{\cdot-}$=84.53mg/ml; EC50.OH=15.7mg/ml) increased with the addition of 20% ($EC50O_2^{\cdot-}$=39.65mg/ml; EC50.OH =13.26mg/ml), 30% ($EC50O_2^{\cdot-}$=25.83mg/ml; EC50.OH=11.8mg/ml) and 40% ($EC50O_2^{\cdot-}$=19.57mg/ml; EC50.OH =10.02mg/ml) of dried plums. The obtained results indicate that the honey with dried plums is a new product with higher biological value comparing to honey.

Keywords: Honey with dried plums; Phenolics; Flavonoids; Radical scavenging activity.

PO-114

Track: *Enabling Technologies*

NOVEL MUSCLE CELL ASSAY FOR IAPPLICATIONS IN BIOMEDICINE

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Loss of muscle mass occurs in aging, a variety of diseases, including cancer, chronic heart failure, aquired immunodeficiency syndrome, diabetes, and renal failure, often aggravating pathological progression. Skeletal muscle atrophy in individuals is associated with diminished quality of life and increased health resource utilisation. Muscle wasting results from an imbalance between protein degradation and synthesis and is enhanced by decreased regenerative repair. Several signaling pathways that may contribute to muscle atrophy have been identified, and there is increasing evidence that oxidative stress, due to reactive oxygen species production plays a role in causing muscle depletion both during aging and in chronic pathological states. Hypercatabolism and in particular, oxidative stress has been proposed to enhance protein breakdown. Moreover, skeletal muscle produces a variety of secreted proteins that have important roles in intercellular communication and affects processes such as glucose homoeostasis. Preventing muscle wasting by promoting muscle growth has been proposed as a possible therapeutic approach where mainly folistatin has been widely used. In the current view, rodents where most of the experiments are performed are far away of being a good model to study the interactions at the cellular and tissue level and to discover new potential candidates in the therapies. Therefore, here, we will describe the cell model of human skeletal cells with the potential applications and impacts.

Keywords: Muscle cells, assay, metabolism, regeneration.

PO-29

Track: *Hot Topics in Natural Products*

GUARANA SEED EXTRACT: GOOD SOURCE OF BIOACTIVE COMPOUNDS

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Guarana (*Paullinia cupana*, Sapindaceae) is a Brazilian native plant, with seeds being the only part suitable for human consumption. The seeds are rich in caffeine, containing up to 8% of the drug, along with small amount of other xanthine alkaloids (theophylline and theobromine) and diferent compounds such as polyphenols, saponins, starch, fats, choline and pigments. The main polyphenols in guarana seeds are catechins. The aim of this study was to investigate the composition of guarana seed extract, obtained by extraction with 60% ethanol, as well as its antioxidant activity against reactive hydroxyl and stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The contents of polyphenols (172.2 mg gallic acid/g) and proanthocyanidins (49.3 mg/g) were determined spectrophotometrically, while the contents of caffeine (128.4 mg/g) and catechins, such as epicatechin (31.13 mg/g) and epicatechin gallate (3.75 mg/g), were analysed by HPLC. The antioxidative activity was measured by electron spin resonance (ESR) spectroscopy. Antioxidant activities of guarana seed extract on hydroxyl and DPPH radicals, expressed as EC50 values, were 0.275 mg/ml and 0.036 mg/ml, respectively. Our results suggest that seed extracts of guarana possess strong antioxidant activity, and they can therefore be used as a natural additive in food, cosmetic and pharmaceutical industries.

Keywords: Guarana seed, poyphenols, caffeine, antioxidant activity.

PO-80

Track: *Neutraceutical Drug Discovery & Therapy*

INHIBITION OF UVB-INDUCED WRINKLE FORMATION AND MMP-9 EXPRESSION BY MANGIFERIN ISOLATED FROM ANEMARRHENA ASPHODELOIDES

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Chronic exposure of human skin to solar ultraviolet (UV) radiation causes photoaging. Naturally occurring phytochemicals are known to have anti-photoaging effects. The present study examined the effect of mangiferin isolated from Anemarrhena asphodeloides on wrinkle formation, skin thickness, and changes in collagen fibers in hairless mice. The *in vitro* effects and possible mechanism of mangiferin on UVB irradiation were determined in human keratinocyte (HEKa) cells. *In vitro* results showed that mangiferin reduced UVB-induced matrix metalloproteinase-9 (MMP-9) protein expression and enzyme activity and subsequent attenuation of UVB-induced phosphorylation of MEK and ERK. In the *in vivo* studies, mangiferin inhibited UVB-induced mean length and mean depth of skin wrinkle based on skin replica, epidermal thickening, and damage to collagen fiber. Taken together, these results indicate that mangiferin exerts anti-photoaging activity in UVB-irradiated hairless mice by regulating MMP-9 expression.

Keywords: Mangiferin, MMP-9, Heka, hairless mice.

PO-165

Tracks: *Drug Metabolism*

PHARMACOKINETIC ANALYSIS AND METABOLITE IDENTIFICATION OF ALOE-EMODIN IN RATS BY LC/MS

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Aloe-emodin is an anthraquinone in rhubarb with various beneficial activities. This study aimed to identify and quantify the metabolites of aloe-emodin in serum after oral and intravenous administrations of aloe-emodin. For the identification of metabolites, serum samples with and without treatment with β-glucuronidase/ sulfatase were deproteinized with methanol and analyzed by LC/MS. The results of LC/MS showed that aloe-emodin was markedly enhanced after hydrolysis with β-glucuronidase/sulfatase. In addition, rhein ([m/z-H] = 283), an oxidized metabolite, was detected in serum before and after hydrolysis either following oral or intravenous dosing of aloe-emodin. Furthermore, the quantitation method of aloe-emodin and rhein in serum was developed and validated. The results showed that aloe-emodin was not absorbed per se, whereas aloe-emodin glucuronides, rhein and rhein glucuronides/sulfates were predominant in the circulation. In conclusion, aloe-emodin was rapidly and extensively metabolized to its metabolites including aloe-emodin glucuronides, rhein and rhein glucuronides/sulfates.

Keywords: Aloe-emodin, pharmacokinetics, metabolism, LC/MS.

PO-41

SYNTHESIS OF AMINO BENZOCYCLOHEPTENE HALIDES FROM NATURALLY OCCURRING BICYCLIC SESQUITERPENES

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Amino benzocycloheptene derivatives are attractive biological targets in theoretical chemistry, pharmaceutical sciences and coordination chemistry [1], and play an important role in the treatment of neurodegenerative, cardiovascular diseases, sarcoma and carcinoma [2]. These have been widely synthesized from commercially available 5-benzocycloheptanone through oxime, azide formation or reductive amination [3].

The essential oil of *Cedrus deodara* is mainly composed of three sesquiterpene hydrocarbons *α*-himachalene, *β*-himachalene, and *γ*-himachalene containing hexahydrobenzocycloheptene as basic skeleton. A new route to high value added compounds synthesized from this inexpensive isomeric mixture of natural products is a challenging task in organic synthesis. Through our recent research, commercially important similar skeleton of amino benzocycloheptene halides (ABHs) were synthesized starting from less valuable isomeric mixture of three himachalenes through two steps consecutive and regioselective process. Scopes of different amino substituents were further investigated successfully. As per earlier reports, in near future ABHs could find potential interest in different area of biological applications for drug design and development targeting antidepressant, antiarrhythmic, analgesic, α-sympathomimetic and anorexigenic activities.

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PO-104

Track: *Recent Advances in Patient Treatment and Care*

ACUTE AND CHRONIC EFFECTS OF ASCORBIC ACID ON THE PHARMACOKINETICS OF CYCLOSPORINE: *IN VIVO* AND *IN VITRO* STUDIES

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Cyclosporine (CsA), an immunosuppressant used for the prevention of allograft rejection in transplant patients, is a substrate of P-glycoprotein (P-gp) and CYP3A4. Supplementation of antioxidant vitamins is not uncommon for transplant patients. This study investigated the acute and chronic effects of ascorbic acid on CsA pharmacokinetics and the underlying mechanisms. Rats were orally administered CsA (2.5 mg/kg) with and without single or multiple doses of ascorbic acid (300 mg/kg). Blood was withdrawn at specific time points and CsA blood concentration was determined by fluorescence polarization immunoassay. For mechanism identification, transport assay of rhodamine 123 in LS-180 and recombinant isozyme of CYP 3A4 were used to evaluate the modulation of ascorbic acid on P-gp and CYP 3A4, respectively. The results showed that both single and multiple doses of ascorbic acid significantly decreased the area under the curve (AUC) and the maximum blood concentration of CsA. In mechanism study, ascorbic acid increased the efflux function of P-gp, whereas CYP 3A4 was not affected. In conclusion, ascorbic acid reduced the bioavailability of CsA through activation of P-gp. We suggest that concurrent intake of ascorbic acid with critical medicines which are P-gp substrates should be avoided to ensure the efficacy.

Keywords: Cyclosporine, P-glycoprotein, ascorbic acid, antioxidant.

PO-97

Track: *CNS Drug Discovery & Therapy*

DECREASED PHENYTOIN BIOAVAILABILITY CAUSED BY COADMINISTRATION OF RHUBARB THROUGH ACTIVATION OF P-gp

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Phenytoin (PHT) is an antiepileptic with narrow therapeutic window. Rhubarb, the rhizome of Rheum palmatum, is an important herb in clinical Chinese medicine. This study investigated the effect of rhubarb on the pharmacokinetics of PHT and the underlying mechanisms.

Rats were orally administered with PHT (200 mg/kg) with and without rhubarb decoction (2 g/kg, 4 g/kg and seven doses of 2 g/kg) in a crossover design. Blood samples were withdrawn via cardiopuncture at predetermined time points and serum concentrations of PHT, PHT glucuronide, 4-hydroxy phenytoin (HPPH) and HPPH glucuronide were determined by HPLC method before and after treatment with glucuronidase. LS-180 cell line was used to evaluate the effect of rhubarb on the efflux of PHT mediated by P-gp.

Our results showed that coadminstration of single dose and multiple doses of rhubarb significantly decreased the Cmax and AUC0-t of PHT, PHT glucuronide, HPPH and HPPH glucuronide. Cell line study indicated that rhubarb increased the efflux of PHT.

In conclusion, rhubarb markedly decreased the oral bioavailability of PHT through activation of P-gp. Patients treated with PHT are suggested to avoid concurrent use of rhubarb to ensure the efficacy of PHT.

Keywords: Phenytoin, rhubarb, pharmacokinetics, P-glycoprotein.

PO-129

STUDY OF LUNG CANCER METASTASES TO THE ADRENAL GLANDS AND TO THE SPLEEN

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Metastases to organs such as the liver, bones or the central nervous system appear to be a frequent complication of malignant lung cancer, whereas metastases to the suprarenal glands are found less frequently [1]. Metastases of lung cancer to the spleen are of great rarity and they are described sporadically [2]. The development of imaging methods increased the number of possibilities to visualize the suprarenal glands. So far, the most frequent indications to diagnose the suprarenal gland tumor are: clinical symptoms and biochemical test results. Currently, we make use of some diagnostic methods, which allow us to visualize adrenal gland and confirm any abnormalities before the clinical symptoms occur [1].

Most often spleen tumor lesions are incidentally detected on imaging tests, requested for another conditions. Primary spleen tumors are of great rarity [3]. Primary cysts acquiring enormous proportions and haemangiomas are classified as benign tumors [4,5]. Metastatic lesions and inflammatory pseudotumors may also be seen, but only very rarely and as casuistic description [6,7].

The authors analyzed 1492 patients with lung cancer. In 12 patients with pathological lesions in the spleen and in 58 in the adrenal glands, fine needle biopsy was performed.

Among those patients, in 58 cases metastases to the adrenal glands and in 11 cases to the spleen were confirmed. All single metastatic deposits in the adrenal glands were located ipsilateral to the primary lung tumor. Metastases in the spleen originated only from the left lung tumor.

In cases with disseminated cancer, 5 patients had confirmed metastases in both adrenal glands, 1 patient with cancer located in the right lung had metastatic lesion in the left adrenal gland.

In these cases metastases to other organs were also detected. The authors came to the conclusion that the direction of the metastatic dissemination depends on the way the cancer spreads. The metastatic deposits, which disseminate via the lymphatic system, tend to locate on the same side (as the primary cancer site) according to the anatomy of the lymphatic system, whereas disseminated metastases seem to be hematogenous.

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PO-85

PRETREATMENT WITH ATRIAL NATRIURETIC PEPTIDE PRIOR TO CORONARY OCCLUSION MIMICS ISCHEMIC PRECONDITIONING(IPC) AGAINST INFARCTION AND ARRHYTHMIAS *VIA* CARDIOMYOCYTE MITOCHONDRIAL K_{ATP} CHANNEL ACTIVATION IN THE INTACT RABBIT HEART DURING ISCHEMIA/REPERFUSION

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Objective(s) & Materials and Methods: To test whether administration of atrial natriuretic peptide(ANP) has cardioprotective and antiarrhythmic effects against ischemia and reperfusion injury in a manner similar to IPC in a well-standardized model of reperfusion arrhythmias in anesthetized adult male rabbits (n=113) subjected to 30 min occlusion of the left coronary artery followed by 120 min of reperfusion.

Results & Discussion: Either one cycle of IPC or ANP(2.5 µg/kg, i.v. bolus) prior to coronary occlusion offers significant infarct size reduction (21.5 ± 1.5 % and 19.6 ± 1.9 %, respectively versus 38.4 ± 3.8 % in saline control group; $P < 0.01$) and antiarrhythmic effects. Both IPC and ANP treatment significantly attenuated the incidence of life-threatening arrhythmias like sustained VT (13 % and 13 %, respectively versus 100 % in saline control group; $P < 0.005$) and other arrhythmias (25 % and 25 %, respectively versus 100 % in saline control group; $P < 0.005$), and increased the number of surviving animals without arrhythmias. 5-HD but not HMR 1883 abolished the beneficial effects of IPC and ANP on reperfusion induced arrhythmias and cardioprotection suggesting that benefits have been achieved via selective activation of cardiomyocyte mitochondrial K_{ATP} channels. ANP-evoked NO release and selective activation of mitoK_{ATP} channels leading to IPC in cardiomyocytes contribute to cardioprotection and antiarrhythmic activity during ischemia-reperfusion in the anesthetized rabbit.

Conclusions: We conclude that administration of ANP peptide prior to coronary occlusion contribute to IPC-like cardioprotective and antiarrhythmic properties in the intact rabbit heart.

PO-57

ANTIMICROBIAL MODULATOR OF INFLAMATORY RESPONSE IN THIRD MOLAR SURGERY COMPARED WITH CONVENTIONAL MEDICATION

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Surgeries performed in retained third molars occur in contaminated field.

This study was to evaluate the differences between conventional medication and topical doxycycline delivered through a gel nanotubes in retained third molar surgery.

A split half mouth, crossed study "*in vivo*" was performed on patients of Hospital Dentistry, Federal University of Uberlândia, Minas Gerais, Brazil, to evaluate pain, bleeding, discomfort, swelling, infection, trismus, and inactive days. We selected 20 patients, which agreed with the research and signed an informed consent term, according to the Ethics Committee in Research. Half mouth was randomly selected to undergo surgery and use of conventional medication (analgesic, anti-inflammatory and antimicrobial) and after 25 to 30 days, the other half of the mouth was subjected to surgery using doxycycline delivered.

In both treatments there was no clinical infection. As for the other evaluations were lower in relation to pain, bleeding, edema, inactive days, trismus and discomfort, when compared with conventional therapy, $P \leq 0.05$ was considered statistically significant ($P \leq 0.05$).

We conclude that the use of doxycycline delivery, was more effective in reducing postoperative signs and symptoms, showing comfort and adherence to therapy, does not interfere with the normal microbiota and reduction in treatment costs.

PO-40

Track: *Hot Topics in Natural Products*

ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF BEETROOT POMACE EXTRACT

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The beetroot pomace, waste product generated primarily during juice processing is also promising source of bioactive compounds (polyphenolic compounds, carotenoids, betalains, vitamins, minerals) which could be used as functional ingredients with antioxidant, antiinflammatory, hepatoprotective, and antitumor properties.

The total phenolic content (45.68 mg GAE/g) was determined according to the Folin-Ciocalteu method. Antioxidant activity of the beetroot pomace extract (BPE) against superoxide anion radicals was evaluated by electron spin resonance (ESR) spectroscopy and the IC50 value was 1.0625 mg/ml. The BPE exhibits cytotoxic properties against Ehrlich carcinoma (EAC) cells *in vivo* due to the induction of oxidative stress. The largest decreases in EAC cell numbers were observed in pre-treated male (approximately 53%) and female (approximately 47%) mice and also EAC cell viability was decreased after administration of the BPE. Together, these observations suggest that pretreatment with the extract interferes with the establishment of tumour growth more significantly than when it is administered simultaneously with the tumour cells implantation. The activities of antioxidant enzymes (SOD, Px) were significantly different between the untreated EAC control group and all groups that were treated with BPE. SOD and Px activities were very low in untreated malignant cells, but increased significantly after administration of the extract.

Keywords: Beetroot pomace extract, antioxidant activity, cytotoxic activity.

PO-110

POSSIBLE ROLE OF AQUAPORINS IN THE PRETERM DELIVERY IN THE RAT

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The aquaporins (AQPs) are a family of integral membrane channel proteins that facilitate rapid passive movement of water. Our aim was to examine the alterations of mRNA expression of the AQPs in the late pregnant and preterm rat uterus. The effects of some uterine-relaxing drugs were also investigated on AQPs expression.

Changes in AQPs mRNA expression was measured by real-time PCR on pregnancy days 18, 20, 21 and 22 in rat uterus and after induction of preterm delivery with mifepristone and prostaglandin E2. The effects of terbutaline or progesterone treatment on AQP5 and AQP8 mRNA expressions were measured in 22-day pregnant rats. The AQP5 and AQP8 mRNA expressions were the highest on days 18-21 which were dramatically decreased on the last day of pregnancy (day 22). In hormonally-induced preterm labor, the mRNA expression of AQP5 and AQP8 markedly decreased similarly to day 22. Terbutaline and progesterone treatment increased the AQP5 and AQP8 expression.

We suppose that decreased expression of AQP5 and AQP8 may have importance in the initiation of delivery in rat. The uterine-relaxing effect is accompanied with the re-expression of these AQPs.

The work was supported by TÁMOP 4.2.1/B-09/1/KONV-2010-0005 grant.

PO-65

MAPPING ENDOCRINE DISRUPTING CHEMICALS (EDCS) USING NETWORK PHARMACOLOGY PRINCIPLES TIED TO HIERARCHICAL CLUSTERING ANALYSIS

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In the recent past a paradigm shift has occurred in biomedical sciences, primarily developed in molecular pharmacology but also involving toxicology and certain other related disciplines. This can succinctly be phrased so that an overall reductionist approach, which places emphasis on individual physiological target proteins and singular effects, has in many respects been replaced by a broader and integrated picture of cellular responses to external stimuli. From an overarching perspective, this is also referred to as a systems biology view. Notably, advancements in analytical technology, experimental technique, multivariate analysis as well as chemo- and bioinformatics have all been instrumental to this view on biological processes, particularly involving cellular responses to chemical perturbation. Another key factor in this scenario pertains to late attrition of many lead drug candidates, translating to major expenditures for developers of pharmaceuticals, which has created an incentive to open new inroads to deepened understanding of chemical-mediated composite responses in cells and organisms. This means that a considerably larger proportion of the target cell/organism complexity, relative to classical molecular pharmacology, is taken into account in the evaluation of biological activity induced by chemical molecules. Essential parts of this perception involve poly-pharmacology (multi-targeting) and network pharmacology (biochemical pathway interaction mapping), the latter being a core concept of this direction. Conceivably, these advancements will entail enhanced opportunities to develop new drugs and to extend therapeutic areas of established medicines, but also new entrances to toxicological risk assessment, particularly in segments known as hazard identification and hazard characterization. In food toxicology, this paradigm shift has hitherto not found much application, but holds great potential to support risk assessment.

We have applied network pharmacology principles to approaching risk assessment of a set of Endocrine Disrupting Chemicals (EDCs), some of which were or still are relatively abundant contaminants of the food and feed chains in many Western areas. This contrivance encompasses two main parts: delineation of cellular pathway-associated topological maps, on the one hand, and the elaboration of a distance map to the remaining chemicals of the set, on the other. The various steps to achieve topological outlines of such cellular interactions involved sequential consultations of several publicly accessible services on the Internet, which each collates an appreciable volume of integrated data from various other serves, as typified by the “Kyoto Encyclopedia of Genes and Genomes” (KEGG), “Online Mendelian Inheritance in Man” (OMIM), Binding DB and Reactome. By means of web-supported services - likewise freely available - listings of key interacting proteins were derived and an ordered protein array was accordingly attached to each chemical. Pairs of such array representations of chemicals were subsequently compared using a special ranking scheme. To further enhance accuracy of these matching exercises an equivalent procedure was applied to “Gene Set Enrichment Analysis” (GSEA) derived pathways and a composite score was eventually derived.

A hierarchical clustering display of results, as derived from optimized multidimensional scale-processed readouts from the aforementioned two-part procedure, revealed relationships within the set of EDCs which largely conforms with current perception of their respective perturbations of mammalian sex-steroid hormone homeostasis. Notably, co-clustering of estradiol with two common environmental contaminants, and two rather potent xenoestrogens, is essentially compliant with literature reports. Joint appearance of some key phytoestrogens, and the phtalate as a singleton, also conforms to anticipation. Conclusively, our quantitative network mapping of EDCs is a novel extension of systems biology generally and network pharmacology in particular and clearly holds potential to enhancing pharmacology evaluation and toxicology risk assessment.

PO-14

Track: *Anti-Cancer Drug Discovery & Therapy*

NOVEL GENISTEIN-LOADED LIPIDIC NANOCARRIER ADJUVANTS ENHANCE ANTICANCER EFFICACY AND OVERCOME CANCER RESISTANCE TO CHEMOTHERAPY

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Genistein, a small, biologically active flavonoid, is found in high amounts in soy, and is best known for its ability to markedly inhibit cancer progression, and metastasis. It has recently emerged as a Pgp drug-efflux pump inhibitor, as well as a potent apoptosis-inducing agent that occurs through disruption of mitochondrial membrane integrity, thus primarily triggering the intrinsic pathway of apoptosis, in different types of malignant cells.

Despite evident anti-cancer activity of genistein, its use is limited by its lipophilic nature, extremely low aqueous solubility, extensive metabolism and poor bioavailability and pharmacokinetics.

Our work describes the development and evaluation of new lipid-based nanocarriers (NCs), namely liposomes (Lip), nano-emulsions (NE) and polymeric phospholipid micelles (Mic), as drug delivery vehicles for improved both oral and parenteral delivery of genistein, via enhancing drug loading, and stability in biological systems. Hence, the therapeutic potential of genistein, either alone or in combination with other chemotherapeutic drugs, e.g. doxorubicin (DOX), against various types of cancers can be established.

Physico-chemically-screened Lips, NEs and Mics loaded with genistein were tested *in vitro* against murine sensitive breast carcinoma (4T1) and resilient colon cancer (C26). Inhibition of drug-efflux in human ovarian (OVCAR) and DOX-resistant (NCI/ADR-RES) cancers cells was assayed fluoromertically using drug-combination ratios with liposomal DOX.

Genistein-loaded vesicles showed high drug solubilization capacity (NE>Mic>Lip) and favorable nano-scale properties, leading to improved delivery of genistein, and subsequent superior cytotoxicity in tested cancers of different origins. Microscopic evaluation indicated enhanced uptake of the Lip drug into target colon carcinoma cells over 4 hours. Moreover, induction of morphological apoptosis features was quite significant after treatment of 4T1 with genistein-loaded Mic, compared to free drug and empty vehicle controls.

Superior cytotoxic effects were demonstrated for genistein Mic and Lip, in both 4T1 and C26 cancer cells, compared to treatment controls. Most importantly, IC50 values for genistein/DOX Lip were at least 3-6 folds < DOX-Lip in NCI/ADR-RES, showing significant synergistic 1:1 drug ratio, and additive 1:2, 2:1 and 3:1 drug ratios, respectively.

Genistein-loaded NCs showed high solubilization capacity and favorable nano-scale properties, leading to improved delivery of genistein to both naïve and drug-resistant cancer cells. These new genistein NCs can lead to therapeutic strategies using this potent pro-apoptotic nutraceutical, either alone or to augment the anti-neoplastic effect of doxorubicin and other susceptible drugs, in resistant tumors over-expressing drug efflux pump.

PO-53

Track: *Hot Topics in Medicinal Chemistry*

SYNTHESIS OF NEW 9-GLYCOSYL-4,9-DIHYDROPYRANO[3,4-B]INDOLE-1(3H)-ONES AS ANTIBACTERIAL AGENTS

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A series of new 9-glycosyl-4,9-dihydropyrano[3,4-b]indole-1(3H)-ones 3 was synthesized in moderate to low yields. 4,9-Dihydropyrano[3,4-b]indole-1(3H)-ones (1) were coupled with different acetobromoglycopyranoses 2 in refluxing toluene in the presence of silver oxide to afford one coupling product of the respective N-glycosides. α-L-

Arabinopyranosides 3j and 3m were the most active glycosides among the tested compounds against certain Gram positive and Gram negative bacterial strains.

Keywords: Pyranoindoles; N-Glycosylpyranoindoles; Antibacterial agents.

PO-125

Track: *Anti-infectives*

COMPARISON BETWEEN REAL-TIME PCR AND ELISA FOR THE DETECTION OF HUMAN CYTOMEGALOVIRUS INFECTION IN RENAL TRANSPLANT PATIENTS IN THE SUDAN

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Objectives: This study was carried out to detect human Cytomegalovirus (HCMV) IgG and IgM antibodies using IgG and IgM ELISA in renal transplant patients in Khartoum state, Sudan and to improve the diagnosis of HCMV through the introduction of Real-time PCR. A total of 98 plasma samples were collected at random from renal transplant patients at Ibin Sina hospital and Salma Centre for transplantation and haemodialysis from August to September 2006.

Results: Among the 98 renal transplant patients, 65 were males and 33 females. The results revealed that, HCMV IgG was present in all patients' plasma 98/98 (100%), while only 6/98 (6.1%) have IgM antibodies in their plasma. The load of HCMV DNA was detected in 32 patients 32/98 (32.7%) with real-time PCR.

Conclusions: The results of the IgG suggested a high incidence of previous infection in all tested groups, while the finding of IgM may reflect a recent infection and reactivation. HCMV detection with real-time PCR in the present study indicated high prevalence among renal transplant patients in Khartoum. In conclusion, the incidence and existence of HCMV in Khartoum State was documented through the detection of HCMV specific antibodies. Further research work should be carried out to characterize HCMV at molecular level.

PO-123

Track: *Cardiovascular Drug Discovery & Therapy*

DIGITAL PHOTO-CONTROL OF THE ENGINEERED CARDIAC TISSUE

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Excitable networks of various origins, such as networks of neural cells, oscillating chemical media, solid state chips exhibiting self-sustained waves (autowaves), etc. always teased computer scientistsas possible prototypes for highly parallel computer processors. Recent development of tissue engineering techniques allows creating and maintaining almost indefinitely networks of excitable cells with desired architecture. As an architectural guide we used nanofibers capable to carry and maintain cardiac cells. The nanofibers allow to align cardiac cells to a desired degree and to make three-dimensional tissue constructs of the desired thickness. One challenging aspect of such systems is creating an interface allowing coupling the network of live excitable cells with a common computer. We are approaching this goal by sensitizing excitable cardiac cells to light and monitoring excitation in them with the aid of fluorescent probes (optical mapping). As a sensitizing substance we use azobenzenetrimethylammonium bromide (AzoTAB). The dark, thermally relaxed, or obtained by irradiation with blue light ($\lambda > 440$ nm) trans isomer of AzoTAB reversibly reduces the occurrence of spontaneous activity, as well as decreases the speed of propagating excitation waves, up to total their suppression. The excitation waves may reactivate after irradiation with near-UV light ($\lambda \approx 365$ nm), which produces prevalence of cis isomer of AzoTAB. AzoTAB-mediated sensitization allows, thus, controlling the excitation waves through the entire cardiomyocyte network either uniformly, or in a preferred spatial pattern.

In order to perform photocontrol of the tissue construct we modified digital projector Taxan KG-PL105S and coupled it with the macroview microscope Olympus MVX-10. The spatial pattern of conducting pathways is created with the aid of computer graphic software and projected on the cardiomyocyte layer. The propagation of excitationis observed and

recorded with the aid of fluorescent Ca^{2+}- or membrane potential dependent dyes. Sensitization of cardiac cells to light combined with optical mapping allows real time interactive photocontrol of heart tissue.

Keywords: Engineered cardiac tissue, AzoTAB.

PO-159

Tracks: *Anti-infectives*

MULTIFUNCTIONAL ACTIVITIES OF SEVERAL POLYOXOMETALATE COMPOUNDS PRESCRIPTION ON RNA VIRUS REPLICATION AND BACILLUS MULTIPLICATION

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Introduction: Polyoxometalates (PM) are discrete oligometric anions of early-transition metal oxides such as tungsten (W), molybdenum (Mo), vanadium (V), antimony (Sb) oxides. Various biological effects of PM have been studied on antitumor, antiviral, and antibacterial activities. For discovery with multifunctional drug in infectious disease, we selected as anti-MRSA, anti-VRSA, and anti-RNA virus what has an effect from these activity compounds, and did mixed prescription.

Materials and Methods: The mixed prescription of PM compounds (MPM) are shown below: (1) V1: VOSO4. (2) V2: K11H(W)3(SbW9O33)2. (3) W1: Na9[SbW9O33]. (4) O1: oxacillin. (5) P1(Irgasan): 5-Chloro-2-(2,4-dichlorophenoxy)phenol. The each PM were synthesized according to literatures and did not chemical reaction their self. The MPM were examined about anti-Influenza virus and anti-constant bacillus activities.

Results: The MPM inhibited Influenza virus replication and constant bacillus multiplication. Further more, the MPM inhibited multiplication of Escherichia coli and fungus also.

PM compounds are act as a direct inhibitor and enhancer of oxacillin or Irgasan.

Conclusion: The MPM are useful as a sterilization antiseptic. Now, the goods which sprayed this MPM on the steamed towel or the paper towel are developed.

Keywords: Polyoxometalate, virus, bacillus.

PO-45

Track*: Inflammation and Immunology*

REAL-TIME QUANTITATIVE AND REPRODUCIBLE MOUSE OSTEOMYELITIS MODEL USING BIOLUMINESCENCE IMAGING TECHNIQUES

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Although osteomyelitis (OM) remains one of the serious problems in a clinical field, there are few animal models for the real-time evaluation of OM. We have established a real-time quantitative mouse OM model using a bioluminescence imaging (BLI) technique, which enables monitoring infection processes from acute to chronic phases with kinetics of immune cells and serum levels of cytokines/chemokines. Bioluminescent strain of *Staphylococcus aureus* was inoculated into the medullary cavity of the femurs of mice. Phosphate buffered saline was administered to control group. Bacterial photon intensity (PI) was sequentially measured by BLI. Serological, flow cytometry, and histological analyses were also performed. Mean PI in OM group reached a peak at 3 days and plateaued at 7 days. Serum levels of interleukin-6 ($P<0.05$), interleukin-1β ($P<0.05$), and C-reactive protein ($P<0.001$) were significantly higher in OM group at 7 days, and serum monocyte chemotactic protein-1 level was significantly higher in OM group at 12 hours compared with control group ($P<0.001$). Ratio of granulocytes in peripheral blood was significantly higher in OM group after day 7 ($P<0.001$). Acute and chronic histological manifestations were observed in OM group. This novel OM model can be a powerful evaluation tool for new antibiotic drugs or bacteria-resistant implants.

PO-46

***Track**: Inflammation and Immunology*

NOVEL BACTERIA-RESISTANT IMPLANT PREVENTS IMPLANT-ASSOCIATED OSTEOMYELITIS

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Current antibiotics and bacteria-resistant implants do not always provide consistent antibacterial effects *in vivo*. We have successfully developed a stable and uniformly-dense silver coating method and obtained significant antibacterial effect both *in vitro* and *in vivo*. Hydroxyapatite (HAp) films were formed on the desired implant, and silver ions were immobilized by immersing the implant coated with HAp films into the special IP6-Ag-containing solution. Silver-coated titanium pins significantly produced growth inhibition zones on LB medium against the bioluminescent strain of *Staphylococcus aureus* (*S.aureus*) than those of control pins ($P<0.001$). In our mouse osteomyelitis model, *S. aureus* was inoculated into the medullary cavity of the femur. Sequential analyses of bacterial luminescence revealed that mean PI in silver group was significantly lower than that in control group at 3 and 7 days ($P<0.01$). Surprisingly, no bacterial signals were detected at 21days in silver group. Serum IL-6 level in silver group was significantly lower at early phase ($P<0.01$), and serum CRP level in silver group became significantly lower after day14 ($P<0.01$) than those in control group. Histological manifestations of osteomyelitis were found significantly less frequently in silver than those in control group. This new silver coating method can be applied to various medical devices.

PO-134

***Track:** Innovative Drug Discovery and Nanotechnology*

NEW PARADIGM OF DRUG DISCOVERY AND DEVELOPMENT

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Details have been discovered previously either by identifying the active ingredient from traditional remedies or by serendipitous discovery. Proper drug target selection and validation are crucial to t he discovery of new drugs.

A new approach based to understand how disease and infection are controlled at the molecular and physiological level and to target specific entities. The process of drug discovery involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy.

Once a compound has shown its value in these tests, it will begin the process of drug development prior to clinical trials. Drug design is a process driven by innovation and technological breakthroughs involving a combination of advanced experimental and computational methods. A broad variety of medicinal chemistry approaches can be used for the identification of hits, generation of leads, as well as to accelerate the optimization of leads into drug candidates.

This addresses these technological advances as well as several new areas that have been created by necessity to deal with this new paradigm, such as bioinformatics, cheminformatics, and functional genomics. With many of these crucial components of future drug discovery now in place, it is possible to devise a critical path for this process that will be used into the new millennium.

Keywords: Bioinformatics, discovery, genomics, leads, target.

PO-109

Track: *Women's Health Drug Discovery and Therapy*

DRUG COMBINATIONS FOR BETTER RELAXATION: STUDIES ON PREGNANT RAT MYOMETRIUM

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Combinations of well-known uterus-relaxing agents may solve the clinical problem of premature birth. Our aim was to investigate the effects of combinations of drugs are already in use in therapy.

Contractions of 22-day pregnant rat myometrial rings (also progesterone pretreated) were investigated in the presence of the terbutaline and nifedipine in isolated organ bath. The pregnancy maintenance effects of drug combinations (salmeterol-progesterone, salmeterol-nifedipine, nifedipine-salmeterol) were also tested on hormonally-induced preterm birth model *in vivo.*

Progesterone pretreatment increased the relaxing effect of terbutaline on isolated uterus and enhanced the pregnancy-maintenance effect of salmeterol. The effect of nifedipine became weaker by progesterone. The combination of terbutaline and nifedipine had strong relaxing effect when terbutaline was given after nifedipine. The pregnancy maintenance effect of nifedipine-salmeterol was almost five times higher as compared with salmeterol-progesterone.

Progesterone enhances the effect of betamimetics, while worsens the action of nifedipine. The combination of betamimetics and nifedipine is very effective, but the administration of terbutaline cannot precede that of nifedipine. Clinical trial for this combination is recommended.

The work was supported by TÁMOP 4.2.1/B-09/1/KONV-2010-0005 grant.

PO-120

IN VITRO AND *IN VIVO* ANTIBACTERIAL ACTIVITY OF ACORN HERBAL EXTRACT AGAINST SOME GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA

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Objectives: The search continues for safe and effective antimicrobial agents, which treat, therapeutically and prophylactically, a wide variety of bacterial infections. This study was undertaken to investigate the antimicrobial properties of herbal extract (acorn) against bacterial pathogens in intestinal tract infections in *in vitro* and *in vivo* conditions and to study the effect of extraction against bacteria in comparison with current antibiotics.

Methods: Ethanol extraction of acorn herb (Jaft) were evaluated against *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeroginosa* in *in vitro* and *in vivo* conditions.

Results: Minimal Inhibitory Concentration (MIC) for each bacterial sample reflected concentration with 10 µg/ml, 10µg/ml, 5µg/ml, 15 µg/ml and 15/µg/ml inhibited the growth of *K. pneumoniae, E. coli, S. typi, S. aureus* and *P. aeroginosa*, respectively. The *in vivo* results showed the infection by *K. pneumoniae, E. coli, S. typi* and *P. aeroginosa* inhibited and the entire infected Rat were treated by the acorn extraction. Control positive rat died after 5 days, while infection rat with *S. aureaus* missed after 8 days.

Conclusion: The finding revealed that acorn extract has great potential as antimicrobial compounds against microorganisms. Thus, acorn extract can be used in the treatment of infectious diseases caused by resistant bacteria especially in gram-positive bacteria.

Keywords: Gram negative-gram positive bacteria-acorn extract.

PO-121

ANTIBACTERIAL ACTIVITY OF LIPPA CITRIODORA HERB ESSENCE AGAINST METICILINE RESISTANCE *STAPHYLOCOCCUS AUREUS*

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The search continues for safe and effective antimicrobial agents with which to treat, therapeutically and prophylactically, a wide variety of bacterial infections. Aims of this study was to determine the effect of Lippia citriodora against Meticiline Resistance *Staphylococcus aureus* (MRSA).

Methods: Lippa Citrofora was tested against *S. aureus* by MIC and disk diffusion methods. MTT assay was used for determination of toxicity of essence.

Results: It was found that the percentage of growth inhibition increasing with increasing concentration steadily up to 55 µl/ml and IC50 value of this assay was 0.245. Minimal Inhibitory Concentration (MIC) for each bacterial sample showed only MRSA inhibited in concentration of 15ul/ml of oil.

Conclusion: The use of plants to heal diseases, including infectious one, has been extensively applied by people. Our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated.

Key words: Lippia citriodora, *S. aureus*, MIC.

PO-7

Track: *Anti-Cancer Discovery & Therapy*

INVOLVEMENT OF PROHEPCIDIN IN THE ANEMIA OF MULTIPLE MYELOMA

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Purpose: Hepcidin is liver-produced peptide implicated in the anemia of inflammation. IL-6 is potent inducer of hepcidin expression. As IL-6 is involved in the pathogenesis of multiple myeloma and serum IL-6 levels are often increased in myeloma patients, we explored the hypothesis that elevated hepcidin expression contributes to the anemia characteristic of this disease.

Materials and Methods: Newly diagnosed patients with Durie-Salmon stage III multiple myeloma patients were enrolled in this study. All patients (median age 61.06 ± 3.1 (41-85) entered the study before receiving any cytotoxic chemotherapy. None of the patients had history of recent infection, blood transfusions or erythropoietin therapy. All human studies were done in accordance with the local regulations. Serum levels of prohepcidin (pHp), Zn-protoporphyrin (ZPP), soluble transferrin receptor (sTfR), C-reactive protein (CRP), ferritin, folate and vit.B12 were measured prior to treatment. After receiving conventional therapy for multiple myeloma in the course of two months studied group underwent second measurement of the above mentioned serum markers. The Student's t test was used to compare normally distributed data. Correlations between the various measured parameters were calculated by Pearson correlation.

Results: Mean baseline levels of pHp were abnormally elevated. pre-treatment hemoglobin levels indicated inverse correlation with pHp and CRP levels*. A statistically significant difference was found between pre-treatment values (1) and after two-month therapy (2): pHp 1/2 (226.06±10.24 /188.81±8.39), HGB 1/2 (96.69±3.24/ 108.19±2.51), CRP 1/2 (3.13±0.12/2.59±0.09), ferritin1/2 (346.25±21.47/ 232,87± 11.48; ZPP 1/2 (102.5±4.56/ 83,56±4.61.

Conclusions: These results indicate that hepcidin is up-regulated in multiple myeloma and may play a role in the anemia of multiple myeloma by both IL-6-dependent and IL-6-independent mechanism. Pharmacologic inhibition of hepcidin expression might exhibit favorable effect on the development of anemia in multiple myeloma patients. Thus, hepcidin lowering agents are a promising new class of pharmacologic drugs to effectively combat the anemia of chronic diseases.

Keywords: Hepcidine, anemia, multiple myeloma, inhibition of hepcidine expression.

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PO-169

DUAL INHIBITION OF CLASSICAL PKC-ALPHA AND PKC-BETA ISOFORMS IN A PHARMACOLOGICAL AND DOUBLE KNOCK OUT MOUSE APPROACH LEADS TO PROTECTION AGAINST EXPERIMENTAL MUNISE DIABETIC NEPHROPATHY

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Background: Both classical PKC isoforms, PKC-alpha and -beta, have been implicated in the pathogenesis of diabetic microvascular diseases. We previously elucidated the specific role of these individual isoforms and revealed that activation of PKC-beta contributes to high-glucose-induced renal fibrosis whereas nephrin and VEGF expression are regulated by PKC-alpha dependent signalling pathway leading to diabetic albuminuria.

Methods: We tested the hypothesis if deletion of both classical PKC isoforms is able to completely abolish the development of diabetic nephropathy in the streptozotocin-induced diabetic mouse model. We studied distinct pharmacological approaches by inhibiting both classical PKC isoforms and validated the phenotype of nondiabetic and streptozotocin-induced diabetic homozygeous PKC-a/b double knock-out mice (PKC-a/b$^{-/-}$) compared to appropriate 129/SV wild type mice.

Results: After 8 weeks of diabetes the high-glucose-induced renal and glomerular hypertrophy and the increased expression of extracellular matrix proteins such as collagen and fibronectin was abolished in the PKC-a/b$^{-/-}$ mice compared to WT controls. Furthermore, the high-glucose-induced expression of the TGF-beta1 was significantly diminished in the PKC-a/b$^{-/-}$ mice compared to diabetic WT mice. The loss of the basal membrane proteoglycan perlecan and the podocyte protein nephrin is prevented in the diabetic state in the PKC-a/b$^{-/-}$ mice. Furthermore, we were able to demonstrate, that a PKC-a/b-inhibitor had a similar effect.

Conclusion: Blockade of the PKC alpha and beta isoforms are able to prevent early diabetic nephropathy while inhibiting prosclerotic glomerular and tubulo-interstitial changes as well as the development of albuminuria. These results demonstrate that down regulation of the dual PKC-isoform activation in the diabetic state *in vivo* is a suitable therapeutic target in the prevention of diabetic microvascular complications such as diabetic nephropathy.

PO-4

Track: *Anti-Cancer Discovery & Therapy*

DESIGN, SYNTHESIS AND EVALUATION OF PHTHALIMIDE/ PYRROLO-δ-CARBOLINE ANALOGUES AS ANTI-NEOPLASTIC AGENTS

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Objective: Thalidomide was withdrawn from the market because of its catastrophic teratogenicity. Currently, this reborn drug is being evaluated in the treatment of multiple myeloma. Structural development of thalidomide led to the development of compounds with a 6-5-6 fused heteroaromatic ring systems which may be effective against various diseases, including multiple

myeloma, AIDS, etc. Considering the biological interest of this drug and its structural development, attempt has been made to design, synthesize some phthalimide analogues and utilise these derivatives for the synthesis and evaluation of some novel pyrrolo-δ-carbolines for their anti-neoplastic activity.

Experimentation: A library of phthalimide analogues structurally related to thalidomide was designed and synthesized. Further, these analogues were utilised to design and synthesize novel pyrrolo-δ-carbolines. All the molecules were characterized by 1HNMR, Mass, and IR. *In silico* evaluation for the synthesized molecules was performed through Schrodinger molecular modelling software using human topoisomerase IIα (hTopoIIα) (PDB id 1ZXM) at the ATP site. The purified compounds were screened for their *in vitro* and *in vivo* antineoplastic activity by MTT Assay and DLA Ascites tumor model respectively.

Results: Molecular modeling studies predicted that the designed molecules bind to the ATP pocket in the ATPase domain of hTopoIIα comparable to Salvicine and ICRF193 which have already been reported as potent competitors of ATP at ATPase domain of hTopoIIα. The *in vitro* and *in vivo* data illustrated that these molecules may function as a potent anti-neoplastic agents.

Keywords: δ-carboline, antineoplastic, phthalimide, *In silico*.

PO-73

Track: *Anti-Infectives*

THE EFFICACY AND SAFETY OF A NATURAL PATENT PENDING COMBINATION OF GINGER AND GOLDENROD ON THE MANAGEMENT OF COLD SYMPTOMS IN COMMUNITY-DWELLING ADULTS: A RANDOMIZED, DOUBLE-BLIND CONTROLLED TRIAL

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Objective: To compare the efficacy and safety of two natural products, a combination of two plant-based extracts (Ginger and Goldenrod), referred to as BDI-630, and a combination of standardized amounts of Echinacea components, referred to as Echinacea, in alleviating cold symptoms in community-dwelling adults.

Design: randomized, double-blind, active-control trial.

Intervention: Patients were required to take BDI-630 (900 mg) or Echinacea (500 mg) twice daily for 10 days.

Subjects: 44 patients, aged between 18 and 60 years old, with new-onset cold symptoms.

Outcome measures: The primary outcome was based on the assessment of the severity of cold symptoms during 14 days by self-report using the Wisconsin Upper Respiratory Symptom Survey (WURSS).

Results: Of the 44 patients enrolled, 39 completed the protocol. Results indicated a significant difference between the two groups: the superiority of BDI-630 over Echinacea was particularly evident between Day 1 and Day 7, as demonstrated by a 7-fold difference. Adverse events (AEs) following BDI-630 were mostly limited to mild gastrointestinal intolerance in less than 10 % of the patients.

Conclusions: BDI-630 (900 mg bid) was shown to be superior to Echinacea (500 mg bid) in alleviating cold symptoms in the adult population. BDI-630 was very well tolerated.

PO-170

CHRONIC PAIN - TRANSLATIONAL MEDICINE WHERE ARE WE NOW?

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Chronic Pain is an important clinical, social and economic problem that has a profound negative impact on the quality of life of a significant proportion of the general population in all countries .In the US the Societal costs of chronic pain is a staggering over 100 billion dollars. The last few decades has seen an expansion in our understanding of the pathophysiology of chronic pain. This was not matched by an increase in the availability of new compounds to meet an area of unmet clinical need. Critical analysis is needed to improve the rate of translational basic science into effective

new drugs. There is still a disproportionate reliance on pre- clinical models, though they may not be highly specific to human pain syndromes. 'Pain heterogeneity' and the lack of innovative classifications that correspond to the clinical picture is a further hurdle. The pharmaceutical industry's obsession with "magic bullet" policy and a single discrete pain target has led to worsening of the attrition ratio. The rising cost of phase II/phase III studies with the higher hurdle of drug registrations has added yet another difficulty. A novel road map is proposed; a multi-layered development plan. where chronic pain characterization is mirrored by the clinical state of a small patient population. Surrogate pain models should be used in reverse translation from patient to human volunteers to animals, Pharmacological validation of the models are used in the same reverse program. The use of a single marker, eg fMRI or a single genotype, is unlikely to lead a significant change in the near future.

PO-87

ROLE OF OXIDATIVE STRESS, INFLAMMATION AND ENDOTHELIAL DYSFUNCTION IN THE PATHOGENESIS OF DIABETIC RETINOPATHY

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Background: Oxidative stress, inflammation and endothelial dysfunction are commonly found in persons with type II diabetes mellitus (DM), but their role in the pathogenesis of diabetic retinopathy (DR) is not fully elucidated. Therefore, the present study investigates the relationship and the role of these factors in the incidence and progression of different stages of DR.

Methods: This study included 85 subjects divided into four groups. First group consisted of 20 healthy subjects who served as controls. The second group consisted of 23 patients with type II DM without retinopathy, while the third group consisted of 20 patients having non-proliferative diabetic retinopathy (NPDR), and finally the last group consisted of 22 patients having severe proliferative diabetic retinopathy (SPDR). For all subjects in all groups, the levels of glycated hemoglobin ($HbA1_c$ %), lipid profiles, malondialdehyde (MDA) and nitric oxide (NO) were measured spectrophotometrically, while tumor necrosis factor-alpha (TNF-α) and soluble Eselectin (sE-selectin) were measured using ELISA technique.

Results: All the above measured parameters were significantly elevated in all diabetic patients with or without retinopathy when compared to control subjects, with the most significant increase in case of the SPDR group, whereas the high density lipoprotein cholesterol (HDL-C) was markedly decreased in all diabetic groups when compared to the control subjects. There was a significant positive correlation between plasma MDA with both TG & $HbA1_c$%, NO & TNF-α and finally s-Eselectin & $HbA1_c$%.

Conclusion: Oxidative stress, inflammation and endothelial dysfunction have a fundamental role in the pathogenesis of DR.

PO-88

CONTRIBUTION OF OXIDIZED LOW DENSITY LIPOPROTEIN AND NITRIC OXIDE IN THE PATHOGENESIS OF EARLY ONSET ACUTE MYOCARDIAL INFARCTION IN EGYPTIAN POPULATION

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Background: There is accumulating data that acute myocardial infarction (AMI) is related to recent onset activation of inflammation affecting atherosclerotic plaques. Increased blood levels of oxidized low density lipoprotein (ox-LDL) could play a role in these circumstances. Inducible nitric oxide synthase (iNOS) is expressed in the myocardium after AMI. Its pathophysiological role in these conditions, however, is not clear. We hypothesized that increased nitric oxide (NO) production from iNOS expression might be one of the factors associated with the incidence of AMI. Thus, the aim of this study was to collect information about the role of oxidative stress, and inflammation as represented by ox-LDL and NO levels, respectively in the pathogenesis of AMI in Egyptian populations.

Methods: The study subjects consisted of 101 age-matched healthy volunteers serving as controls and 104 AMI patients. Serum ox-LDL levels were determined quantitatively by ELISA, while serum NO levels were measured calorimetrically by Griess method.

Results: The serum levels of both ox-LDL and NO were significantly elevated in the AMI patients when compared to control subjects ($P \leq 0.005$, $P \leq 0.0001$) respectively. In addition, there was a significant positive correlation between serum ox-LDL and NO levels in the AMI patients ($P = 0.001$).

Conclusion: Our results revealed the association of oxidative stress and inflammation, as represented by ox-LDL and NO respectively, with the incidence of AMI.

**This study was supported financially by the Science and Technology Development Fund (STDF), Grant No. 2951.*

PO-95

Track: *CNS Drug Discovery & Therapy*

IDENTIFICATION OF POTENTIAL TREATMENTS FOR POLYQ-MEDIATED NEURODEGENERATIVE DISEASE SCA17 WITH TBP TRANSGENIC MICE AND MOUSE CEREBELLAR CULTURE SYSTEMS

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Spinocerebellar ataxia type 17 (SCA17) is an autosomal dominant neurodegenerative disease results from expanded CAG repeat of TATA binding protein (TBP) gene. The CAG repeat expansion encodes polyglutamine (polyQ) stretch in the N-terminal of mutant TBP protein, which causes nuclear aggregation and cell degeneration of the neurons. SCA17 transgenic mice overexpressing human TBP-109Q in the cerebellum was generated and have ataxia phenotype. To evaluate the potential therapeutic compounds for SCA17, we established cerebellar primary culture and organotyptic slice culture as drug screening platform. Compared to the cultures from wildtype mice, the SCA17 mouse cerebellar primary culture showed reduced neurite growth, and the slice culture showed extensive nuclear aggregation. Several traditional Chinese herbal medicine and synthetic compounds were first evaluated with SCA17 cerebellar primary and slice culture to identify whether drugs could improve the neurite morphology and reduced the TBP aggregation. Potential drugs were then applied to the SCA17 mice and several neurobehavioral and neuropathological characterization were conducted to verify their therapeutic effect *in vivo*.

Keywords: SCA17, transgenic mice, cerebellum, primary culture, slice culture.

PO-39

Track: *Hot Topics in Natural Products*

NONI JUICE SIGNIFICANTLY INCREASED THE SYSTEMIC EXPOSURE OF METHOTREXATE IN RATS

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Methotrexate (MTX), a dicarboxylate, is an important immunosuppressant with narrow therapeutic index. Clinically important pharmacokinetic drug-drug interactions exist between MTX and non-steroidal anti-inflammatory drugs, which may result in bone marrow suppression and acute renal failure. The transport of MTX was associated with multi-drug resistance proteins (MRPs), organic anion transporters (OATs) and breast cancer resistance protein (BCRP). Noni, the fruit of Morinda citrifolia, is popularly used as a health food worldwide. The major constituents of noni are phenols including scopoletin, rutin, lignans and anthraquinones. The conjugated metabolites of phenols, which are putative substrates of MRPs, OATs or BCRP, may compete with MTX for these anion transporters. This study investigated the acute and chronic effects of noni on MTX pharmacokinetics in rats. Rats were administered MTX with and without single or seven doses of noni juice (6 mL/kg). Blood was withdrawn at specific time points and serum MTX concentration was determined by fluorescence polarization immunoassay. Our results showed that coadministrations of single dose and multiple doses of noni significantly increased the AUC0-t, Cmax and MRT of MTX. In conclusion, concomitant intake of noni juice may alter the efficacy or toxicity of MTX.

Keywords: Methotrexate (MTX), multi-drug resistance proteins (MRPs), organic anion transporters (OATs), breast cancer resistance protein (BCRP).

PO-48

ESTABLISHMENT OF A NONINVASIVE QUANTITATIVE MOUSE MODEL OF SOFT TISSUE INFECTION USING BIOIMAGING

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The infection of multi-drug resistant bacteria such as MRSA remains a serious problem. There are only a few soft tissue infection animal models in which the quantity and distribution of bacteria can be traced. We have established a noninvasive quantitative mouse model of gluteus muscle (GM) infection using bioimaging (BI). A bioluminescent strain of MRSA was inoculated into the GM of BALB/c adult mice. The bacterial photon intensity (PI=photons/sec/cm^2/steradian) was then sequentially measured by BI. Serological and histological analyses were also performed. Bacterial signal was detected at GM just after inoculation of MRSA. Mean PI plateaued at approximately 7days (1.52pprox x 10^5 PI) and could be stably measured until 4weeks. Serum levels of interleukin-6 and C-reactive protein were significantly higher at 7days. Histological analysis revealed that the muscle necrosis and cumulated neutrophils with bacterial colonies were observed in the GM at 7days. We have successfully visualized and quantified the bacterial growth in a mouse GM model using BI, which enabled us to monitor the infectious process without euthanizing the animals. This model can be useful for assessing the effect of novel antibiotics or antibacterial implants.

PO-81

Track: *Anti-Cancer Discovery & Therapy*

CHEMICAL CONSTITUENTS FROM CYTOTOXIC EXTRACT OF *CROTON LAEVIFOLIUS*

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Several species of Croton has been used by many nations in the world as traditional medicine for various illnesses. The phytochemical investigations on the chemical constituents of Croton species around the world have resulted in the discovery of some new compounds and many of them showed various, promising biological activities especially on the terpenoid type of compounds. A bioassay-guided study on the stem barks of Malaysian *Croton laevifolius* was initiated for their new and bioactive chemical constituents. The hexane, dichloromethane and methanolic crude extracts were screened for cytotoxicity on various human's cancer cell lines e.g. lung cancer cell line (A549), prostate cancer cell line (PC-3), melanoma cell line (A375), Colon cancer cell line (HT-29) and normal liver cell line (WRL-68) where the hexane extract was found cytotoxic against A549 (EC50 48.3). The hexane crude extract was fractionated and two fractions were screened as cytotoxic, namely fraction E and fraction F. Fraction E was found cytotoxic against A549 (EC50 45.02), PC-3 (EC50 47.48), A375 (EC50 44.79) and WRL-68 (EC50 48.14) while fraction F showed cytotoxicity against A549 (EC50 15.89), PC-3 (EC50 40.21) and WRL-68 (EC50 36.47). Further work on isolation and purification on the cytotoxic fractions gave six triterpenes and a mixture of steroidal triterpenes; stigmasterol and β-sitosterol. These initial findings showed the potential of this plant in searching for the biologically active compounds.

Keywords: Cytotoxic, chemical constituents, *Croton laevifolius*.

PO-152

Track: *Nutraceutical Drug Discovery & Therapy*

BETTER LIFE PROGNOSIS WITH NEONATAL INTENSIVE MANAGEMENT IN TRISOMY 13

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The policies for the treatment of trisomy 13 in our hospital, the provision of active intensive treatment including resuscitation and surgery according to the clinical conditions of an infant and in accordance with the wishes of the infant's parents. We retrospectively studied the clinical data of 16 patients with trisomy 13, admitted to NICU at our hospital from 1989 to 2010. None was diagnosed trisomy-13 before birth; 9 were delivered by C-section and oxygen was administered to all patients during postpartum resuscitation. Mechanical ventilation was used in 9 patients after tracheal intubation and tracheotomy was performed in 2 patients. Regarding prognosis, 9 patients died, 3 were referred to another hospital, and 4 were discharged from the hospital. Four and 7 patients died within 7 and 30 days after birth, respectively. Nine patients survived for >1 month, 7 for >180 days, and 5 for >3 years. Median survival for 16 patients was 733 days. The patients with trisomy 13 who received intensive treatments survived longer compared to the previous data. This study provides important information concerning genetic counseling with trisomy 13 not only for their parents but also physicians.

PO-93

Track: *Nutraceutical Drug Discovery & Therapy*

NUTRACEUTICAL INHIBITORS OF PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1) IN DIABETES THERAPY

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Obesity is a global health problem and a gateway to diabetes. Plasminogen activator inhibitor (PAI-1) is progressively overexpressed in both conditions. PAI-1 is the fast acting and specific inhibitor of tissue (tPA) and urokinase (uPA) plasminogen activators that regulate proteolysis. In obesity and diabetes it has been linked to the increased incidence of thrombosis. However, PAI-1 is also involved in the regulation of other proteins engaged in hemostasis. These molecules include tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), transforming growth factor β (TGF-β), and angiotensin II, all of which up-regulate PAI-1 in various cell types or can be up-regulated by PAI-1. PAI-1 plays a critical role in the insulin resistance syndrome, which leads to type 2 diabetes mellitus, and is associated with its side effects such as an increased risk of diabetic nephropathy, atherosclerotic cardiovascular disease and others. Analyzing many natural compounds on its ability to inactivate PAI-1 in human plasma we have found unexpectedly that some ingredients of black tea have strong inhibitory activity against PAI-1. Among four tested theaflavins, theaflavin-3'-gallate was the most potent in PAI-1 inhibition followed by theaflavin-3,3'-digallate, the other two i.e., theaflavin and theaflavin-3-gallate did not show inhibitory activity.

PO-143

Track: *Diabetes and Obesity Drug Discovery & Therapy*

ENHANCED CD4+CD25+ REGULATORY T CELLS YIELD WITH SPLENIC PROLIFERATION AND PROTECTION AGAINST OXIDATIVE STRESS BY NICOTINAMIDE IN DIABETIC PREGNANT RATS

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Maternal diabetes is one of the most common complications during pregnancy. Metabolic changes in diabetic pregnancy affect fetal development and fetal glucose homeostasis. Several complications of diabetes may be related to increased intracellular oxidants and free radicals formed. The present study was initiated to evaluate the effects of nicotinamide on cd4+cd25+ regulatory T cells (treg) yield, production of reactive oxygen species (ros) by neutrophils, proliferation of splenocytes and the MRNA levels of two antioxidant genes, Cu/Zn superoxide dismutase (sod) and catalase (cat)). 50, 100 and 200 mg/kg nicotinamide were supplemented p.o to mated (pregnant diabetic) rats from day 6 to 20 of gestation. Flow cytometry analysis showed an increased expression of treg with nicotinamide supplementation. Increased proliferation of splenocytes was seen in both resting and lipopolysaccharide (lps) stimulated cells. However, there was reduction in oxidative burst activity of neutrophils in response to phorbol myristate acetate (pma) and *E. coli* activation. More over the up regulation in the MRNA expression of sod and cat which were quantified with real-time qrt-pcr indicates its antioxidant effect. In summary, nicotinamide enhanced the immune system through stimulation of adaptive immune cells namely lymphocytes and treg and decreasing ROS production by neutrophils and provides protection against oxidative stress. These findings provide evidence for the usage of nicotinamide as a supplement or as adjunct to therapeutic agents in diabetic pregnant individuals with weakened immune system.

Keywords: Nicotinamide, qrt pcr, splenocytes, treg cells, diabetic pregnant rats.

PO-66

Track: *Translational Medicine*

ACCULTURATION AND SELF-REPORTED HEALTH AMONG HISPANICS USING A SOCIO-BEHAVIORAL MODEL: THE NORTH TEXAS HEALTHY HEART STUDY

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Background: Acculturation is a continuous, firsthand contact with other cultures functioning at both group and individual levels and is reflected in our culturally diverse society, calling for a greater understanding of the environmental and cultural impact on health. Self-reported health (SRH), a robust and well validated predictor of future mortality for all racial/ethnic groups, has been differentially reported by Hispanics compared to whites, especially based on their acculturation status. This study investigated the relationship between acculturation and SRH among Hispanics. An adapted Andersen framework was used to develop logistic regression models to assess for an association between acculturation and general health status.

Methods: Hispanic participants (n = 135), as part of the North Texas Healthy Heart Study, were administered standardized questionnaires on acculturation, psychosocial measures which included sense of control, stress, depression and social support and a single item SRH measure. In addition, physiological measurements and demographic characteristics including age, gender, body mass index, medical history, and socioeconomic status were also obtained.

Results: Bivariate analyses found Mexican-oriented participants 3.16 times more likely to report fair/poor SRH compared to Anglo-oriented Hispanics. Acculturation was also associated with SRH in multiple regression models controlling for enabling, need, and predisposing factors together (OR: 3.53, 95% CI: 1.04, 11.97).

Conclusions: Acculturation status was associated with SRH after accounting for other underlying factors. Medical and public health professionals should promote the use of acculturation measures in order to better understand its role in Hispanic behaviors, health outcomes and health care use. Such research findings will contribute to the design of culturally sensitive prevention and treatment strategies for diverse and immigrant populations.

Grant Number P20MD001633 from the National Center on Minority Health and Health Disparities

Reference:

Johnson, K.L., Rodriguez, M., Carroll, Fulda, K.F., J. Cardarelli, K. & Cardarelli, R. (2010). Acculturation and self-rated health among Hispanic adults. BMC Public Health, 10:53.

PO-156

FABRICATION OF UNIFORM-SIZED LIPID VESICLES WITH BIO-INSPIRED STENCIL WITH MICROFLUIDIC DEVICE

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Lipid vesicles have attracted extensive interest because they can be encapsulated biological solution. Upon encapsulating reactive agents, these vesicles can be modified into biosensors to respond to a variety of environmental stimuli and bioreactors to mimic molecular responses. Lipid vesicles are commonly fabricated by the bulk hydration of lipids in aqueous buffer, freeze-thaw cycling, film hydration, reversed phase evaporation and extrusion methods.However, these methods are often reading to heterogeneous and uncontrolled size during lipid vesicle formation process.

Recently, droplet and deep channel based microfluidic systems have demonstrated versatility not only in processing and sampling reagents but also in the encapsulation of biological substances in droplets.In these methods, microfluidic systems allow for precise control of mixing via molecular diffusion with reproducible and controlled mechanical fluid force over micrometer length scales. Nevertheless, microfluidic systems are required of complex designed channel for precise size control of lipid vesicles and excessive washing step to reuse.

In this study, we developed fabrication system of lipid vesicles with bio-inspired stencil with microfluidic device, uniformly. We obtained super uniformity lipid vesicles compare with other microfluidic systems, which are controlled flow velocity and channel designed. Therefore, this device could be implemented for point of care drug encapsulation process.

PO-114a

Tracks*: CNS Drug Discovery & Therapy*

KR-33028 ATTENUATES ISCHEMIA/HYPOXIA-INDUCED BLOOD-BRAIN BARRIER HYPERPERMEABILITY THROUGH MAINTAINING TIGHT JUNCTION INTEGRITY

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We previously demonstrated that KR-33028, a novel inhibitor of Na+/H+ exchanger-1 (NHE-1), exerts neuroprotective effects during cerebral ischemia. In the present study, we investigated whether KR-33028 elicits cerebrovascular protective effects against ischemia-induced blood-brain barrier (BBB) dysfunction in vivo and in vitro. Effects of KR-33028 on ischemia/hypoxia-induced BBB dysfunction in vivo were evaluated by examining brain water content, Evans blue extravasation and tight junction alteration in a rat cerebral ischemia model. In vitro effects of KR-33028 on ischemia/hypoxia-induced BBB dysfunction were investigated by determining permeability of Evans blue-albumin or [14C] sucrose, tight junction alteration in brain microvessel endothelial cells (bEnd.3 cells). In addition, the intracellular Ca2+ level were measured using an imaging technique. KR-33028 inhibited the activation of NHE-1 induced by intracellular acidosis in bEnd.3 cells. KR-33028 ameliorated brain edema in rat brain ischemia model. KR-33028 also ameliorated BBB hyperpermeability and disruption of occludin and ZO-1 induced by ischemia/hypoxia in vivo and in vitro. Furthermore, KR-33028 prevented hypoxia-induced subcellular redistributions of occludin and ZO-1 in vitro. Hypoxia-induced increases in intracellular Ca2+ levels in vitro were reduced by KR-33028. These findings suggest that KR-33028 ameliorates BBB hyperpermeability and TJs alteration during ischemia/hypoxia, and that KR-33028 has the therapeutic potential to prevent BBB dysfunction after brain ischemia.

Keywords: Na+/H+ exchanger-1, blood-brain barrier, tight junction proteins, ischemia/hypoxia.

PO-27

Track: *Drug Delivery & Targeting*

CAFFEIC ACID PHENETHYL ESTER ACTIVATION OF NRF2 PATHWAY IS MEDIATED BY COVALENT BINDING TO KEAP1

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Caffeic acid phenethyl ester (CAPE) is a polyphenolic natural product that possesses numerous biological activities including anti-inflammatory properties and CAPE-mediated Nrf2 activation is likely to be responsible for its biological effects. We investigated chemical biology of CAPE activation of Nrf2. CAPE, which has two electrophiles, catechol and Micheal reaction acceptor (MRA), in it, was chemically modified to afford CAPE derivatives with either each or no electrophile, which was subjected to experiments to examine cellular Nrf2 activity. While CAPE and a CAPE derivative with catechol moiety, dihydroxydihydrocaffeic acid phenethyl ester (DHHC), but not the other derivatives, activated Nrf2 pathway. Biotin labeled CAPE derivatives with catechol moiety, but not the other derivatives, precipitated Keap-1 when they were incubated in cell lysates followed by addition of streptavidin beads. Moreover, CAPE activation of Nrf2 and Keap-1 binding of the biotin labeled CAPE derivatives were enhanced substantially by chemical oxidation of them.

PO-21

Track: *Drug Delivery & Targeting*

A NOVEL STRATEGY FOR ORALLY ACTIVE PEPTIDE THERAPY FOR TREATMENT OF INFLAMMATORY BOWEL DISEASE: COLON-TARGETED CELL-PERMEABLE NFκB INHIBITORY PEPTIDE AMELIORATES EXPERIMENTAL COLITIS

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Background & Aims: For the purpose of development of orally active peptide therapeutics targeting NFκB for treatment of inflammatory bowel disease (IBD), two major barriers in oral delivery of therapeutic peptides, metabolic lability and tissue impermeability, were circumvented by introduction of a colon-targeted delivery system and cell permeable peptides (CPP) to NFκB inhibitory peptides (NIP).

Methods: Suppression of NFκB activation was compared following treatment with various CPP conjugated NIPs (CPP-NIP). The most potent CPP-NIP was loaded in a capsule coated with a colon specific polymer, which was administered orally to colitic rats. The anti-inflammatory activity of the colon-targeted CPP-NIP was evaluated by measuring inflammatory indices in the inflamed colonic tissue. For confirmation of the local action of the CPP-NIP, the same experiment was done after rectal administration. Tissue permeability of the CPP-NIP was examined microscopically and spectrophotometrically using FITC-labeled CPP-NIP (CPP-NIP-FITC).

Results: NEMO binding domain peptide (NBD, TALDWSWLQTE) fused with a cell permeable peptide CTP (YGRRARRRARR), CTP-NBD, was most potent in inhibiting NFκB activity in cells. Colon-targeted CTP-NBD, but not colon-targeted NBD and CTP-NBD in an enteric capsule, ameliorated the colonic injury, which was in parallel with decrease in MPO activity and the levels of inflammatory mediators. Intra-colonic treatment with CTP-NBD alleviated rat colitis and improved all the inflammatory indicators. CTP-NBD-FITC was detected at much greater level in the inflamed tissue than was NBD-FITC.

Conclusion: Introduction of cell permeability and colon targetability to NIP may be a feasible strategy for an orally active peptide therapy for treatment of IBD.

PO-23

A COLON SPECIFIC PRODRUG OF CELECOXIB HAS A POTENTIAL TO IMPROVE PHARMACOLOGICAL AND TOXICOLOGICAL PROPERTY OF CELECOXIB FOR PROPHYLACTIC TREATMENT OF COLORECTAL CANCER

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To develop a colon specific prodrug of celecoxib, a Cox-2 selective inhibitor, that could improve cardiovascular toxicity and therapeutic effectiveness for chemoprevention of colorectal cancer, aspart-1 or 4-yl celecoxib (A1C and A4C), succinyl celecoxib (SC) and N-succinylaspart-1 or 4-yl celecoxib (SA1C and SA4C) were prepared and evaluated as a prodrug with such beneficial properties. On incubation with the small intestinal contents, while SC, SA1C and SA4C were stable, A1C and A4C were degraded to liberate celecoxib. In the cecal contents, the other conjugates except for SC and SA4C were cleaved to release celecoxib. These results suggest colon specific delivery and activation of SA1C. On oral administration of SA1C or celecoxib, no SA1C was detected in the blood and urine indicating the limited absorption of SA1C. SA1C delivered a much greater amount of celecoxib to the large intestine while keeping the plasma concentration of celecoxib at much lower level, which is consistent with no change of the serum level of 6-keto-$PGF_{1\alpha}$ whose decrease is associated with the cardiovascular toxicity of celecoxib. Moreover, SA1C administered orally supplied a greater concentration of celecoxib for the whole colonic tissue. Taken together, SA1C may be a colon specific prodrug of celecoxib with improved therapeutic properties.

PO-140

Track: *Pharmaceutical Research & Development*

DESIGN AND FORMULATION DEVELOPMENT OF PARACETAMOL LOLLIPOPS FOR CHILDREN

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Drug dosage forms have become a costly affair for third world countries. Especially in Africa region pediatric mortality rate is more due to one of the factor is economy and affordability of individuals. Hence the present work has been takenup to protect the interests of child health which is nations pride. Most of the illnesses are associated with fevers, headache and body aches and it is very difficult for parents to manage pediatric patients to administer the drug dosage forms as most of the formulations are bitter in nature. In the present investigation, an attempt has been made to formulate medicated sugar based tablet lozenges (Lollipops) for kids using Paracetamol as model drug, which is commonly used analgesic, anti-pyretic and anti- inflammatory. There are many dosage forms available in the market but still there is a need for new palatable dosage forms which acts effectively. So the present investigation has been taken up to design, prepare and evaluate hard candy based medicated lozenges (Lollipops) to meet the need of improved action dosage form. The benefits of these prepared lozenges are increase in bioavailability, reduction in gastric irritation by passing of 1^{st} pass metabolism thereby increase in onset of action. The candy based 125 mg Parcetamol lozenges (Lollipops) were prepared using sucrose and liquid glucose (Corn Syrup) as base with a mucoadhesive polymer Na CMC (Sodium Corboxy Methyl Celulose) in varied ratios to increase the retention time of the dosage form for atleast 30 minutes. All the formulations prepared were subjected to various physico- chemical parameters such as hardness, drug content uniformity, weight variation, moisture content etc. The prepared formulations have a hardness of 11-12 Kg/cm^2, not gritty, mouth feel freshness taste. Stability studies of selected formulations were also carried out at 37^oC and 45^oC for a period of six months. Formulations were tested for drug excipient interactions subjecting to IR Spectral analysis and reports revealed undisturbed drug peaks in formulations. *In-vitro* drug dissolution studies showed 97.62% for F_0 in 15 Minutes, 65.04% for F_1, 78.01% for F_2 and 85.42% for F_3 release of drug in 30 minutes. The hard boiled candy lozenges(Lollipops) can provide an attractive alternative formulation in the treatment of pain and fever in pediatrics. This is the need based drug delivery system for the pediatric patients especially when the patients are unable to cooperate the treatment eg. burncases, accidents, asthma etc.

Keywords: Paracetamol, Lollipops.

PO-51

DEVELOPMENT, EVALUATION OF NANOPARTICLES AND HERB–DRUG INTERACTION STUDY OF *GYMNEMA SYLVESTRE* EXTRACT ON THE PERSPECTIVES OF PHARMACOKINETICS

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Purpose: Quality, safety and efficacy are major bottlenecks in the herbal drug development like identification and authentication of raw drugs, batch reproducibility, physico-chemical stability, adulteration and contamination. Another major issues associated with herbal drug development are poor bioavailability of bioactive constituents and herb-drug interaction, which affects both the safety as well as efficacy of the therapy. Therefore, the present work has been undertaken to address major issues like quality control, herb-drug interactions and bioavailability studies on important Indian traditional medicine, *Gymnema sylvestre* as a case example official drug mentioned in Indian Pharmacopoeia and Ayurvedic pharmacopoeia and Indian Herbal Pharmacopoeia for the treatment of diabetes.

Experimental: In this research we developed analytical method for the quantitative estimation and identification of gymnemagenin using HPLC-ESI-MS/MS method in *Gymnema sylvestre* extract and its marketed formulations. In order to overcome the poor bioavailability issue of *Gymnema sylvestre*, we have developed natural polymeric based nanoparticles of *Gymnema sylvestre* extract using chitosan by ionic gelation method. *Gymnema* delays the absorption of other drugs when taken simultaneously and shows additive effect on diabetes when taken with synthetic antidiabetic drugs, in concern with this report we have designed objective to study the herb–drug interaction of *Gymnema sylvestre* extract on the perspectives of pharmacokinetics of synthetic antidiabetics.

Results: The developed method was validated based on ICH-Q2B guidelines and was found to be accurate, precise and linear over a relatively wide range of concentrations (5-300ng/ml). This method can serve as a useful quality control tool and biomarker in pharmacokinetic studies as well for *Gymnema sylvestre* and its formulations. The mean particle size of optimized nanoparticles was 122 nm, zeta potential 51.07mv and polydispersity index was 0.325 with globular in shape (SEM studies). Prepared nanoparticles were further evaluated for entrapment efficiency and drug release which showed 80% of drug entrapment with 95.12% of drug release for 16 hrs. Herb–drug interaction of *Gymnema sylvestre* extract on the perspectives of pharmacokinetics of synthetic antidiabetics is undergoing in our laboratory.

PO-36

IN VITRO ANTICANCER ACTIVITY OF *ABUTILON INDICUM* LEAVES ON HUMAN BREAST CARCINOMA CELL LINE (MDA-MB-231)

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OBJECTIVE: *Abutilon indicum*Linn. (Family: Malvaceae) commonly called as 'Country mallow'(English), 'Kanghi'(Hindi) and 'Atibala'(Sanskrit), is generally used in Ayurveda, Siddha and Unani Medicinal systems. The plant is distributed as a weed in India, Sri Lanka and topical regions of America and Malaysia. The various parts of *A. indicum* are documented to possess various medicinal properties like hepatoprotective, wound healing, anti-diarrhoeal, analgesic, antimalarial, antifertility, hypoglycemic, immunomodultaory, antimicrobial and anti-inflammatory. The present work, explores the anticancer activity of crude methanolic extract of *A. indicum* leaves (MEAI) and its sub-fractions.

METHOD: MEAI and its sub-fractions, obtained by partition separation using chloroform (MEAI-C) and ethyl acetate (MEAI-E), were tested on human breast carcinomacell lines (MDA-MB-231) along with normal cell line (HEK 293) by MTT [3- (4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide] assay at different concentrations (20-60µg/ml). The absorbance was read at 590 nm on a multi-well plate reader (Victor3^{TM}, Perkin Elmer). Percentage inhibition of proliferation was calculated against control (without sample).

RESULTS AND CONCLUSION: A decrease in the growth of cancer cells was observed with increasing concentrations of MEAI, MEAI-C and MEAI-E. The crude MEAI showed 47.62% growth inhibition at 60µg/ml. While, the MEAI-C showed aninhibition of 40.53% at 60µg/ml, MEAI-E showed 46.94% inhibition at the same concentration. The results were compared with a reference compound, Quercetin, which showed 31.16 % growth inhibition at 60µg/ml. The study disclosed a highly significant *in vitro* anticancer effect of leaves of *A. indicum*, which must be further exploited for the development of a potential therapeutic agent for breast cancer.

PO-2

Track: *Academic CRO/Industrial collaborations in drug discovery*

IMPROVED PRODUCTION OF SOME IMPORTANT ANTI- UROLITHIASIS ACTIVE INGREDIENTS FROM *ARBUTUS POVARII* LIBYAN MEDICINAL PLANTS BY MODERN BIOTECHNOLOGY

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This work aims to study the over expressed genes related to the anti-lithotopic monoterpenoid indole alkaloid (MIA) pathways (pathway engineering from the medicinal plant shemeri, *Arbutus povarii* (L.) by modern biotechnology. In the present study, *in vitro* cell system-based as well as the recombinant DNA technology was utilized for both studying and producing important anti-kidneystone secondary metabolites that are likely to be manipulated and manufactured from plantlets recovered through cell cultures. The main approach relies on the production of plantlets, through tissue culture after being treated with chemical elicitors (auxins, cytokenins, nitric oxide or precursors).

Plant seeds of this important medicinal plant were collected. As for *in vitro* regeneration research a cell culture system was established to recover homogenous, pathogen-free, environmentally-independent plantlets at a regular automated rate. The effects of different types and concentrations of auxins and cytokinins [ex. 6-Benzylaminopurine (BA), kinetin, thidiazuron (TDZ), combinations of kinetin with NAA, BA with indole-3-acetic acid (IAA) or NAA, BA with 6-(g - Dimethylallylamino) purine (2ip) or TDZ], the effects of two exogenous substrates (e.g., tryptophan and loganin as precursors of Shikimate and Mevalonate pathways) as well as the plant-signaling elicitor (e.g., nitric oxide or NO) will be studied on the recovered plantlets.

Molecular analyses to indicate presence (through polymerase chain reaction or PCR) and overexpression (through northern blotting) of the induced genes in the treated plantlet leaves. Plantlets recovered from the two biotechnological approaches will be tested on different levels as Activities of enzymes involved in the biosynthesis pathways of such anti-lithiotrpic monoterpenoid indole alkaloids (MIA) in cell cultures.

Keywords: Anti-Urolithiasis, *Arbutus povarii*.

PO-25

OPTIMISATION OF LIPOSOME-ENCAPSULATED CYCLIC DIPEPTIDES

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Purpose: Experimental design using response surface methodology (RSM) is an effective way to optimize various processes and formulations. Using ANOVA analysis, investigators are able to determine how formulation parameters influence the performance of the formulation by assessing interaction effects in order to obtain an optimum formulation. Cyclic dipeptides have been shown to inhibit tumour cell growth in various cancer cell lines but problems with their physicochemical characteristics such as solubility and cell permeability have limited further development of these compounds as clinically effective anti-cancer agents. The aim of this study was to optimize a liposome-based drug delivery system incorporating cyclo(His-Gly) and cyclo(His-Ala) using statistical experimental design and response surface methodology.

Methods: Liposomes, containing various compositions of phosphatidylcholine, cholesterol, stearylamine, vitamin E and polyethylene glycol$_{2000}$-conjugated phosphatidylethanolamine were prepared according to the thin film hydration method and extruded through 100 nm pore polycarbonate filters. Cyclo(His-Ala) or cyclo(His-Gly) was passively encapsulated

into liposomes by including them in the hydration solution. Free drug was separated from encapsulated drug by passing liposome suspensions through Sephadex G50 columns. A 2^{5-1} fractional factorial design was used to screen various factors, after which, RSM utilizing a central composite rotatable design (CCRD) was used to assess the influence of stearylamine content and cholesterol content on encapsulation efficiency, cellular uptake, membrane leakage, polydispersity and zeta potential.

Results: A quadratic mathematical model was used to fit data for all responses and all correlated well with this model. The most significant interaction between cholesterol and stearylamine content was noted for the response of cellular uptake, indicating that these two formulation parameters can not be altered independently of each other. Statistical optimization of the formulation yielded a stable liposome formulation with an average encapsulation efficiency of 0.123 mg drug/mg lipid.

PO-26

ANTICANCER ACTIVITY OF FOLATE-TARGETTED LIPOSOMAL CYCLIC DIPEPTIDES

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Purpose: Targetting of folate receptors is a viable means of selectively delivering chemotherapeutic agents *in vivo* as folate receptors are often found to be over expressed in tumour cells. This enable the development of site specific drug delivery systems for the treatment of various cancers. The use of surface modified nanoparticles such as liposomes with targeting agents such as antibodies has been well investigated. However, problems associated with immune responses in the treated individual could limit this approach clinically. Modifying the surface of a liposome to include a molecule such as folic acid, which is widely distributed throughout the body, may minimize recognition of targeted liposomes by the reticuloendothelial system (RES) while still targeting cells over expressing folate receptors. This approach was used to develop targeted liposomes encapsulating cyclic dipeptides (CDPs) for the potential treatment of cancer, as CDPs have been shown to inhibit tumour cells.

Methods: The folic acid conjugated lipid, folic acid polyethylene $glycol_{2000}$ cholesteryl hemisuccinate (F-PEG-CHEMS) was synthesized from PEG_{2000}-*bis*-amine, cholesteryl hemisuccinate, folic acid and N-hydroxysuccinimide. Liposomes were prepared according to the thin film hydration method were lipid films were formed by dissolving phosphatidylcholine, cholesterol, vitamin E, stearylamine and F-PEG-CHEMS in a 9:1 choloroform:methanol mixture and evaporated in a rotary evaporator overnight. Films were hydrated with a buffered solution containing either cyclo(His-Ala), cyclo(His-Gly) or carboxyfluorescein at 50 ℃ for three hours and extruded through 100 nm polycarbonate filters. Free and encapsulated drugs were separated using gel exclusion chromatography. The cytotoxic potential of free and encapsulated drug on HeLa and MCF-7 cells was then determined using a standard cytoxic assay were cells were cultured in normal and folate-free RPMI medium.

Results: Encapsulation of CDPs in folate receptor targeted liposomes by incorporating F-PEG-CHEMS into the phospholipid bilayer as a ligand showed a significant improvement for all drugs and in all cell lines tested, particularly for HeLa cells grown in low folate media. This indicates that cyclic dipeptides, particularly poorly lipophilic molecules, are ideal candidates for inclusion into targeted drug delivery systems such as liposomes. Long term storage for folate tethered liposomes was, however, shown to be limited resulting in particle aggregation after a week at room temperature. Further investigation on the reasons for this instability is therefore required.

PO-116

A NOVEL POTENT ANTIMICROBIAL PEPTIDE AS A POTENTIAL TOPICAL AGENT

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With the rapid rise in the incidence of wound infections caused by multidrug-resistant microorganisms, many researches for new topical antibiotics have been extensively performed to overcome the development of the multidrug-resistant microorganisms. Antimicrobial peptides (AMPs) are one of the most promising alternatives of new antibiotics against the multidrug-resistant strains, because their mechanisms of antimicrobial action differ from those of conventional antibiotics. We designed a novel AMP, NKC, composed of two repeats of helix structures flanked by helix-capping

motifs in both N- and C-termini as a possible topical antibiotic drug (Park *et al.*, 2004). The peptide showed a broad-spectrum of antimicrobial activity without cytotoxicity against most of microorganisms, including multidrug-resistant strains in the range of the minimal inhibitory concentration (MIC) values (0.25~1 μg/ml). In addition, the wound healing effect of NKC has been explored for human keratinocyte cells (HaCaT). NKC strongly activated migration of HaCaT during an *in vitro* wound healing process, involving in the transactivation of epidermal growth factor receptor (EGFR), and the effect was maximized at 2 μg/ml of NKC. Our results indicate that the antimicrobial peptide, NKC has a great potential as a topical agent.

Reference:

Park *et al.* (2004), JBC 279(14): 13896-13901.

PO-77

Track: *Process Chemistry and Drug Manufacturing*

NEW AND EFFICIENT SYNTHESIS OF ARIPIPRAZOLE BY THE REDUCTIVE ALKYLATION OF AMINES PROCEDURE

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Aripiprazole (7-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butoxy}-3,4-dihydro-1H-quinolin-2-one is approved psycho-tropic drug. We report the results of our investigations of synthesis of aripiprazole, by the reductive alkylation of amines procedure. This procedure allows the conversion of a carbonyl functionality to an amine group by treatment of a mixture of a carbonyl compound and an amine with a suitable reducing agent. In our case, the aldehyde, obtained by hydrolysis of the corresponding acetal (7-(4,4-dimethoxybutoxy)-3,4-dihydro-1H-quinolin-2-one) was used as the carbonyl compound, while 1-(2,3-dichlorophenyl)piperazine was used as the amine. Sodium triacetoxyborohydride was applied as the reducing agent. In this synthese the aripiprazole was prepared from commercially available reagents and were isolated in very good yield (93%) and purity (>99%). Despite the fact that this method is often used in academia and industry, to date the preparation of aripiprazole by the reductive alkylation of amines procedure has not been described, neither in scientific literature nor in patents.

Keywords: Aripiprazole, Reductive Alkylation of Amines, Triacetoxyborohydride.

PO-105

Track: *Process Chemistry and Drug Manufacturing*

CONFORMATIONAL STUDIES OF THE SALICYLAMIDE DERIVATIVE BELONGING TO THE ARYLPIPERAZINE CLASS OF SEROTONIN RECEPTOR LIGANDS

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In our long standing research of serotonin receptor ligands from arylpiperazine class, we have recently found 2-{3-[4-(2-methoxyphenyl)piperazin-1-yl]propoxy}benzamide (1) as the parent member of the cyclic and acyclic derivatives of arylpiperazinesalicylamides studied [1]. Binding affinities of 1 for 5-HT1A and 5-HT7 receptor sites were Ki = 21 nM and 234 nM, respectively, and were the highest in the group of salicylamides investigated. The biological properties of compound 1 were elucidated using molecular modeling procedures [2].

In the search for potentially bioactive conformation of 1 we examined its conformational properties in solid state and in solution by crystallography and NMR methods respectively, as well as we simulated its most preferable conformations in vacuum, in water and at receptor binding sites by means of molecular modeling techniques.

In the present study, computer aided conformational studies of 2-{3-[4-(2-methoxyphenyl)piperazin-1-yl] propoxy}benzamide (1) is discussed, in comparison to X-ray analysis of monocrystals and 2D 1H-1H NMR.

Keywords: Serotonin receptor ligands, arylpiperazine, conformational studies, salicylamide.

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PO-139

MANAGEMENT OF PATIENTS WITH CHRONIC VIRAL HEPATITIS BY IFN-ALPHA – ASPECTS OF FIBROGENESIS AND THE ROLE OF IL-10

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In patients with Chronic hepatitis B (VHB) and C (VHC) we investigated the effect of therapy with IFN-α on serum hyaluronic acid (HA) - a biochemical marker of liver fibrosis and the relationship of the IL-10 pattern to virological therapeutic response.

IL-10 was investigated before the treatment (MO), after 1 month of the treatment (M1) and at the end of the treatment (MX).

Serum HA in MO significantly correlated with the extent of liver fibrosis. We observed statistically significant decrease in HA in all patients (responders /R/ and non-responders /NR/ as well in MX.

All patients with VHB and R in VHC group had significantly higher pre-treatment IL-10 levels, when compared to controls.

A constant decrease in IL-10 was observed in VHB R subgroup, reaching the significant difference only in MX.

In VHC patients, in the R subgroup a significant decrease in IL-10 levels was observed in M1, while an increase was observed in NR subgroup. Serum HA reflects the stage of fibrosis. Serum IL-10 is a response-predicting marker in antiviral therapy.

PO-138

Track: *Translational Medicine*

THE ROLE OF IL-10 IN DETERMINING THE RESPONSE TO ANTI-TUMOR NECROSIS FACTOR ANTIBODY TREATMENT IN CROHN´S DISEASE

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In patients with Morbus Crohn (MC) before and after treatment with anti-TNF antibody (infliximab) in Month 1 and 5 (Mo 1, Mo 5) were investigated: CDAI, interleukin 10 (IL-10), acute phase proteins /AFP/ (alpha-1 antitrypsin, alpha-1 acid glycoprotein, haptoglobin), proteosynthetic liver function parameters /PFP/ (prealbumin and cholinesterase) and plasma levels of conjugated dienes (CD).

Levels of all AFP were significantly lowered in Mo 1, PFP increased in Mo 1. The levels of CD in Mo 1 decreased in 60% and in Mo 5 in 74% of patients.

Patients with a decrease in CDAI more than 50% in Mo 1 remained stable and had a significant decrease in IL-10 levels in Mo 1 and in Mo 5 as well. In patients with a drop of CDAI less than 50% in Mo 1 the clinical activity raised and a significant increase in IL-10 levels was observed in Mo 1 and 5 as well. The effect of infliximab showed the down-regulation of several components of the inflammatory cascade in patients with MC.

PO-68

Track: *Anti-infectives*

QSAR MODELING OF ANTIMICROBIAL ACTIVITY OF SOME 2-SUBSTITUTED BENZIMIDAZOLE DERIVATIVES

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Because of their excellent activities, benzimidazole and its derivatives have a long history as antimicrobial agents. Several thousands of benzimidazole analogs have been synthesized and screened for pharmacological activity. They are of wide interest because of their diverse biological activity and clinical applications. These heterocyclic systems have different activities as they can act as bacteriostats or bactericides, as well as fungicides and they are present in numerous antiparasitic, antiprotozoal and antiviral drugs [1-3]. Some of 2-substituted benzimidazole derivatives were confirmed to have a moderate *in vitro* anticancer activity, as well as some of them exhibited antitumor activity against human hepatocellular carcinoma (HEPG2), human breast adenocarcinoma (MCF7) and human colon carcinoma (HCT 116) cell lines [4].

In continuation of our studies on inhibitory activities of benzimidazole derivatives [5-7], in the present work we examined the activity of different substituted 2-methyl and 2-aminobenzimidazoles against Gram-negative bacteria *Escherichia coli* and studied the quantitative effect of molecular structure on inhibitory activity. The main objective was to establish a quantitative structure-activity relationships (QSAR) and derive a high-quality model which would link the structure of these compounds with their inhibitory activity.

The validity of the model has been established by the determination of suitable statistical parameters. The established model was used to predict inhibitory activity of the benzimidazoles investigated and close agreement between experimental and predicted values was obtained. The low residual activity and high cross-validated r2 values (r2CV) observed indicated the predictive ability of the developed QSAR model. It indicates that the antibacterial activity of series of 2-substituted benzimidazole derivatives can be successfully modeled using different molecular descriptors.

Keywords: Benzimidazole, antibacterial activity, quantitative structure-activity relationship, *Escherichia coli.*

Acknowledgement

These results are part of the project No. 172012, supported by the Ministry of Science and Technological Development of the Republic of Serbia.

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PO-124

SYNTHESIS AND ANTITUMOR ACTIVITY OF SOME NEW XANTHOTOXIN DERIVATIVES

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The condensation of 4-amino-9-methoxy psoralene (4-aminoxanthotoxin) with some aromatic aldehydes led to the formation of 4-arylimine xanthotoxin derivatives **2a**–**h**, which were cyclized with mercaptoacetic acid to afford the thiazolidinone derivatives **3a**–**h**. On the other hand, the reaction of aminoxanthotoxin **1** with some anhydrides afforded 4-imidione derivatives **3a**–**d**. When **1** reacted with some isothiocyanates, the thiourea derivatives **5a**–**c** were obtained but

the thiourea derivative **6** was obtained when **1** reacted with ammonium thiocyanate. The thiourea derivative **6** was cyclized by the reaction with monochloroacetic acid in the presence of sodium acetate to give aminothiazolidinone derivative **7**, but when the same reaction is carried out in the presence of pyridine, the thioxoimidazolidinone **8** was formed. The condensation of xanthotoxin sulphonamide with aromatic aldehydes gave the aryliminosulphonyl derivatives **9a**–**e**. Xanthotoxin sulphonyl hydrazine condensed with some anhydride afforded sulphonic acid imide derivatives **10a**–**c**. The antitumor and cytotoxic activities of **9** synthesized derivatives were tested, five compounds were found to be active, they inhibited the growth of HeLa cells.

PO-115

AN EFFICIENT siRNA DELIVERY SYSTEM FOR CANCER THERAPY USING NOVEL CELL-PENETRATING PEPTIDES

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Small interfering RNA (siRNA) has emerged as one of the new therapeutic strategies for various diseases due to its target-specific gene silencing. However, its relatively high molecular weight, negative charge, and low stability limit its *in vivo* applications. To overcome these problems, cell-penetrating peptides (CPPs) consisting of 10-30 amino acids with a net positive charge have been intensively explored as an siRNA delivery vehicle. The peptide-mediated delivery system has many advantages, such as high efficiency of delivery into mammalian cells, no gene mutation, and the ease of preparation. In this study, we designed novel CPPs, named BR2, BR3, NKC, and NRC, based on the structure of buforin (Park *et al*., 2000). These peptides with cancer-cell specificity showed high cell penetration efficiency. Of these peptides, BR2 showed the highest cellular uptake of siRNA, similar to that of PEI in HeLa cells. These peptides formed stable complexes with siRNA by an electrostatic interaction. The CPP-siRNA complexes were <300 nm in size and non toxic to normal cells. Besides, the complexes were resistant to degradation by ribonucleases. Therefore, it seems that our BR2-mediated delivery system constitutes a promising strategy for an siRNA delivery into cancer cells for therapeutic purposes.

Reference :

Park *et al.* (2000), PNAS 97(15): 8245-50.

PO-94

THERAPEUTIC STRATEGIES TARGETING CHAPERONE AND PROTEASOME FOR POLYQ-MEDIATED SCA

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In polyQ-mediated disorders, the expansions of translated CAG repeats in the disease genes result in long polyQ tracts in the respective proteins, leading to intranuclear and cytoplasmic accumulation of aggregated polyQ proteins inside neurons. The molecular chaperones act in preventing protein misfolding and aggregation. Induction of ubiquitin proteasome also enhances the clearance of aggregate-prone proteins. To screen compounds enhancing chaperone/proteasome function for effective treatment of polyQ diseases, a HEK-293FT cell line with mCherry, ZsYellow1 and AmCyan1 fluorescent reporters driven by heat shock transcription factor 1 (HSF1), heat shock cognate protein (HSPA8) and heat-inducible HSP70 chaperone (HSPA1A) promoters, respectively were used as a basis for screening compound enhancing chaperone function. Cells expressing a short destabilizing sequences fused to the COOH-terminus of green fluorescent protein (GFP^u) was used as a basis for screening compound enhancing proteasome function. Through high-content screening, synthetic compounds and Chinese herbal medicines were identified for their ability of enhancing chaperone and/or proteasome function. In retinoic acid-induced differentiated SH-SY5Y cells expressing SCA17 TBP/$Q_{36\sim79}$-GFP, the expressed TBP/$Q_{61\sim79}$-GFP formed aggregates, accompanying with reducing neurite outgrowth. These TBP lines are currently used to assess the potential therapeutic strategies for enhancing chaperone or proteasome function.

PO-108

SCREENING OF TRADITIONAL CHINESE HERBS (TCHs) FOR ESTROGENIC ACTIVITIES AS ALTERNATIVE TO HORMONE REPLACEMENT THERAPY (HRT) AND THE EFFECTS OF TCHs ON BREAST CANCER RISK

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Hormone replacement therapy (HRT) is widely used for releasing the menopause symptoms, including hot flushes, urogenital symptoms, sleep problems, osteoporotic fracture and colorectal cancer. However, increased exposure to hormones increases our risk of being diagnosed with cancer, especially breast cancer. Phytoestrogens are plant-derived compounds found in a wide range of TCHs, which show much lower affinities for the estrogen receptors comparing to that of estrodiol. They are widely marketed as an alternative to estrogen replacement therapy. It is still unclear whether phytoestrogens affect breast cancer risk, since studies are not in agreement. Therefore, screening of TCH with estrogen activities, and study their effects on breast cancer is crucial. To this end, we used transgenic medaka developed in our lab, which is sensitive to estrogenic substances to screen the TCHs for estrogenic activities. Among 32 herbs and 38 active ingredients we screened, herb Fructus Psoraleae, and its active ingredients bakuchiol showed estrogenic activities. How Fructus Psoraleae and bakuchiol affecting the breast cancer risk were evaluated with estrogen receptor positive breast cancer cell line MCF-7 and estrogen receptor negative cell line MDA-MB-231. Also, how they worked through the estrogen-dependent pathway was demonstrated with *in vivo* model.

PO-117

A NOVEL CELL-PENETRATING PEPTIDE, BR2, FOR THE EFFICIENT DELIVERY OF A scFv INTO CANCER CELLS

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Cell-penetrating peptides (CPPs) have drawn special attention as one of the alternative intracellular drug delivery vehicles. Even though various CPPs have proven very effective in the delivery of different therapeutics, there are some concerns about non-specific penetration and cytotoxicity of CPPs. Therefore, cancer-specific and non-toxic CPPs are strongly needed for effective cancer treatments. In this study, we designed several derivatives with cancer cell specificity based on the cell-penetrating motif of an anticancer peptide buforin IIb (Lee *et al*., 2008). One of the derivatives, BR2 was cancer-specific and cytotoxic only to cancer cells while non-toxic to normal cells. The cellular uptake of BR2 was concentration dependent and affected by temperature and ATP contents of cells. Moreover, BR2 showed the higher translocation efficiency than a well-known CPP Tat (49-57). To study the capability of BR2 as an intracellular carrier, BR2 was fused to a 28-kDa EGFP and a single-chain variable fragment (scFv) directed toward a mutated K-ras (G12V), respectively. Both EGFP and scFv fused to BR2 were effectively delivered into HeLa cells. Furthermore, the BR2-scFv induced a higher degree of apoptosis than a Tat-scFv in K-ras mutated HCT116 cells. These results suggest that a novel cell-penetrating peptide BR2 has potency as a useful drug delivery carrier with cancer specificity for the cancer treatments.

Reference

Lee *et al*. (2008), *Cancer Lett.*, **271(1)**: 47-55.

PO-50

HYBRID SENSORS AND REGULATORS OF POLYOL COMPOUNDS

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Development of polyol-responsive materials gives impulsion to construct self-regulating drug-delivery systems. Smart hybrid materials which change their physicochemical properties with external stimuli by the presence of polyol compounds, included polyphenols and glucose, were prepared.

Materials are based on alanine or insuline entrapped into a silica matrix functionalized with an aminophenyl boronic acid derivative.

It was firstly synthesized 3-acrylamidophenylboronic acid (APBA). A silica matrix was prepared by sol-gel. During ageing gel APBA and alanine or insulin was added.

It was pointed out that alanine or insuline is embedded in the silica. In contrast, APBA interacts strongly with silanol groups from silica. Hydroxyl groups bonded to B atoms in APBA interact with OH groups from polyphenols or glucose present in aqueous media changing the compactness of silica-APBA-alanine (insuline) material, as revealed by the measurement of fractal dimension. Then alanine or insulin leaches, reacting with phenols or glucose, respectively.

PO-6

Track: *Anti-Cancer Discovery & Therapy*

IDENTIFY THE SERUM METABOLIC FOR DRUG REVERTANT OF METHOTREXATE RESISTANCE NPC-TW01 AND HONE-1 CELL LINES

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Nasopharyngeal carcinoma (NPC) is one of the predominant cancers found in the southern part of Asia including Taiwan, Hong Kong, Fukien and Singapore. Clinically, methotrexate (MTX) is the first line treatment for nasopharyngeal carcinoma cells (NPC). Unfortunately, cancer cells can quickly develop drug resistance during the treatment, suggesting that the MTX uptake and/or metabolism machineries might be impaired. Therefore, how to stop or reverse the cancer cells develop drug resistance become a very important topic for cancer treatment. In order to investigate the possibility of reverse MTX-resistance by co-treatment with certain Traditional Chinese Medicine (TCM), several highly MTX-resistant human nasopharyngeal carcinoma cell lines, NPC-TW01 and HONE-1, have been developed and characterized in our laboratory. In the present study, we found that s several flavonoid serum metabolite isolated from rat which orally feed with flavonoid-rich TCM can significantly reverse the MTX-resistance. These results suggest that those TCM-metabolites might play an important role to re-sensitize the MTX-resistant cancer cell he detail mechanisms of reverse the MTX-resistance are active investigation in our laboratory.

Keywords: Methotrexate, Traditional Chinese Medicine (TCM), Drug Revertant.

PO-99

DESIGN OF SMART PEO-PPO-PEO – MAGNETIC DRUG DELIVERY SYSTEM FOR ALZHEIMER'S DISEASES DIAGNOSIS AND THERAPY

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The precise mechanism underlying the Alzheimer's disease (AD) is not fully understood. However, recent reports suggest that smaller, soluble amyloid-β (Aβ) oligomers are more likely to be the pathogenic agents of disease. Indeed, a large number of inhibitors with differing targets and mechanisms of action are available for the treatment or affect the rate of progression of AD. Nevertheless, challenges remain due to the fact that drugs that achieve therapeutic levels may not penetrate the blood brain barrier (BBB) in sufficient concentrations to exert a desired effect. Temperature-responsive magnetic block copolymers showed transition temperature in the range of 25 - 39 ^{o}C. The magnetic cores compose of conjugated Congo Red (CR) maghemite (CR-Fe_2O_3) nanoparticles (NPs) have a great advantage as multimodal imaging agents, while superparamagnetic CR-Fe_2O_3 also possesses the hyperthermia therapy function. Block copolymer shell has the role of the drug carrier whilst attached by antibody provides targeting tools or navigation system. Obviously there are so many interactions between constituents who can affect the structure and function of drug delivery system (DDS). Therefore, the structure–property relationship is investigated by DLS, TEM, FTIR, TGA and SAXS. Results showed conjugation of nanoparticles and loading of drug change the size of micelles and transition temperature range of polymer. Thus tuning the contribution of constituents is essential in designing of successful DDS.

Keywords: Alzheimer's disease, drug delivery system, amyloid-β, PEO-PPO-PEO copolymer, magnetic polymers.

PO-130

PALM OIL-DERIVED TOCOTRIENOL AS THE NEXT GENERATION ANTI-CANCER VITAMIN E

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Tocotrienols (T3) is now considered to be a promising anti-cancer agent due to its potent effects against a wide range of cancers. A growing body of evidence suggests that in addition to its anti-oxidative and pro-apoptotic functions, T3 also possesses other anti-cancer properties. These include the inhibition of epithelial-to-mesenchymal transitions, the suppression of VEGF tumor angiogenic pathway and the induction of anti-tumor immunity. More recently, my lab has demonstrated that T3 has chemosensitization and anti-cancer stem cell effects, further demonstrating the potential of T3 as an effective anti-cancer therapeutic agent. Research has now focused on testing T3 as the next generation vitamin E for chemoprevention and cancer treatment. This presentation will summarize our recent on the anti-cancer effects of T3 and our current progress in setting up the first clinical trial for testing the anti-cancer effect of T3 against prostate cancer.

PO-135

Track: *CNS Drug Discovery & Therapy*

A NOVEL PLANAR PATCH-CLAMP MICROCHIP FOR INTERROGATING SYNAPTIC ACTIVITY IN NEURONS

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The interrogation of cellular activity at the level of the ion channel is important to understanding basic physiology and pathophysiology of all excitable cells-especially brain cells. Currently, the premier technique for investigating ion channels is glass electrode patch clamping. However, this technique is technically challenging and time intensive. In an attempt to automate this process, much research has gone into using planar patch-clamp chip technology. However, up until now, this approach has only allowed the interrogation of suspended cells, and not the physiologically relevant model achieved through cultured neurons. Thus, our ability to test neurons that are connected in networks-a key model for understanding neurological disorders and subsequent pharmaceutical screening of potential drugs-remains limited. We have now developed a novel form of planar patch clamp technology that allows, for the first time, a low-noise, high-resolution interrogation of cultured Lymnaea neurons with the resolution to measure synaptic activity.

Results: We show that both whole-cell currents and action potentials can be recorded on a planar patch clamp surface over an extended period of time on cultured cells. These neurons form tight seals with the chips as demonstrated by our ability to perfuse a fluorescent dye into the individual neuron through the subterreanean channel. Further, our study shows the first ever recordings of synaptic currents as well as synaptic plasticity related changes in neurons cultured in a network configuration as detected on a planar patch clamp chip.

Conclusions: Having the capacity to study ion channel activity on a planar patch clamp chip allows us to move into an entirely new field of biomedical research. On the medical science front, the ability to interrogate ion channel activity over an extended period of time has great implications for studying synaptic development and neuronal changes, a keystone of neuroscience that is relatively untouched. On the pharmaceutical development front, this technique will now allow for medium throughput, high information drug screening for ion channel diseases, which had previously been a very slow process.

Keywords: Planar Patch Clamp, Drug Screening.

PO-1

Track: *Academic CRO/Industrial collaborations in drug discovery*

MEASURING ADHERENCE TO ANTIRETROVIRAL THERAPY IN NORTHERN TANZANIA: FEASIBILITY AND ACCEPTABILITY OF THE MEDICATION EVENT MONITORING SYSTEM

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Background: An often-used tool to measure adherence to antiretroviral therapy is the Medication Event Monitoring System (MEMS), an electronic pill-cap that registers date and time of pill-bottle openings. Despite its strengths, data can be compromised by inaccurate use and acceptability problems due to its design. These barriers remain, however, to be investigated in resource-limited settings. We evaluated the feasibility and acceptability of using the MEMS caps to monitor adherence among HIV-infected patients attending rural clinic in Tanzania's Kilimanjaro Region.

Methods: Eligible patients were approached and asked to use the caps for three consecutive months. Thereafter, qualitative, in-depth interviews about use of the caps were conducted with patients. The caps data were used to corroborate the interview results.

Results: Twenty-three of the twentyfour patients approached agreed to participate. Apart from use on travel occasions, patients reported no barriers regarding caps use. Unexpectedly, the caps design reduced patients' fear for HIV-status disclosure. Patients indicated that having their behavior monitored motivated them to adhere better. Caps -data showed that most patients had high levels of adherence and there were no bottle-openings that could not be accounted for by medication intake. Non-adherence in the days prior to clinic visits was common and due to the clinic dispensing too few pills.

Conclusions MEMS- Caps use was readily accepted by patients. Although used accurately by most patients, patients need to be more explicitly instructed to continue use the cap when travelling. Even HIV-clinics with sufficient staff and free medication may impose structural adherence barriers by supplying an insufficient amount of pills.

Keywords: MEMS Cap, feasibility, acceptance, ART users, northern Tanzania.

PO-70

Track: *Anti-infectives*

SPECIES IMMUNITY AND PROSPECTS OF PREVENTING INFECTIOUS DISEASES OF PLANTS, ANIMALS, AND HUMANS

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All attempts at developing the methods for prevention of infectious diseases, including viral ones, are aimed at disrupting the natural interspecific relationships between microorganisms and susceptible species, as, e.g., in vaccination, which employs the mechanisms of acquired immunity; hence the impossibility of preventing diseases that entail immune system failure, such as AIDS. This also precludes the development of methods for preventing infections in plants, which, in contrast to animals, lack the immune system and whose infectious diseases annually cost millions of dollars in damage, e.g., to American agriculture in the 1970s. Other disadvantages of vaccination are that postvaccination immunity is not inherited and that, according to the active susceptibility concept, the large-scale vaccination has led and will lead to the appearance of new infectious diseases [1]. It is important, therefore, to search for new possibilities to prevent infectious diseases. In this regard, the active susceptibility hypothesis may be of interest because it allows the development of a novel method for preventing plant, animal, and human infectious diseases, including AIDS, based on species immunity mechanisms. An application for patent on the concept of such a method has been published by WIPO [2]. This method, in contrast to routine vaccination, is natural, reliable, and hereditary and is expected to be free of the risk of health complications and the appearance of new infectious diseases.

Keywords: Infection, prevention, plants, animals, humans, HIV.

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PO-133

THE GUANINE-CYTOSINE CONTENT INFLUENCE THE EVOLUTION

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The first is downloading "build" of the human genome sequence, which comprises some Mb of DNA, files containing sequence data and the associated GenBank. Annotation files were downloaded from National Center for Biotechnology Information. For each segment, the average GC content at nongenic DNA not masked by Repeat Masker was calculated. Alignments containing more than 20 kb of repeat sequence were retained for further analysis. Some suggestion of scientists they worked in this trend that the repeated sequences might be caused by substitutions occurring in site repeats prior to insertion. Using **analysis of Human Single-Nucleotide Polymorphisms** (SNPs) **method within site under studying repeats** was identified from the GenBank 'build" annotation files. Using **statistics,** confidence intervals (**CIs**) for correlation coefficients relating patterns of substitution and other sequence characteristics were estimated using nonparametric bootstrapping. All site subtypes are **GC** rich (average GC content of site studying sequences and are relatively rich in CpG sites. The analysis detected that the GC-rich regions include many genes with short introns while **GC**-poor regions are essentially deserts of genes. This suggests that the distribution of **GC** content in mammals could have some functional relevance. On the other hand, the sexes also differ in the relative rates of crossover and gene conversion.

Keywords: GC content- DNA files, GenBank-Mutation

PO-47

***Track:** Inflammation and Immunology*

CELL-CYCLE DEPENDENT PHOSPHORYLATION OF AIRE PROTEIN EXOGENOUSLY EXPRESSED IN HeLa CELLS

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Essential roles of Aire within thymic epithelial cells in the medulla (mTECs) for establishing self-tolerance have recently been highlighted by studies using Aire-deficient mice, manifesting organ-specific autoimmunity. Nevertheless, biochemical studies for AIRE protein have been hampered by the lack of cell lines constitutively expressing AIRE. In order to approach this issue, we have established HeLa cells stably transfected with GFP-human AIRE fusion gene. As expected, we observed GFP expression from the nuclei as nuclear dot, as is for Aire within mTECs. Interestingly, we found that nuclear dot formation by AIRE was dependent on the cell cycle; AIRE nuclear bodies disappeared during mitosis, and they quickly re-appeared after cell division. Concomitantly, AIRE protein extracted from the cells enriched for mitosis showed a slower mobility compared with that extracted from asynchronized cells. We reasoned this change of the mobility of AIRE as the phosphorylation of AIRE protein during the mitosis. We speculate that dynamic nature of AIRE protein we observed might be physiologically relevant to the function of AIRE in order to develop the drugs for the treatment of the disease.

Keywords: Autoimmunity; Aire; phosphorylation; cell cycle.

PO-10

SYNERGISM FROM COMBINATION OF DESIGNED *TRANS*-PALLADIUMS WITH PHYTOCHEMICALS IN HUMAN OVARIAN TUMOUR MODELS

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Ovarian cancer remains an ongoing challenge as tumour relapses in many patients due to acquired or intrinsic resistance. Combination therapy using drugs with different mechanisms of action can offer a means of overcoming drug resistance. Since *trans*-palladium compounds containing sterically hindered ligands are sufficiently tumour active and phytochemicals are increasingly reported to sensitize (or kill) tumour cells to chemotherapeutic agents by modulating various cellular pathways leading to chemoresistance, we have hypothesized that combinations of *trans*-palladiums with phytochemicals may act synergistically. In this study, binary combinations of four *trans*-palladium compounds coded as EH4, TH5, TH6, and TH7 and three phytochemicals thymoquinone (TQ), epigallocatechin gallate (EGCG) and ursolic acid (UA) have been applied to A2780, A2780^{CisR}, and A2780^{ZD0473R} ovarian cancer cell lines using three different sequences of administration: 0/0 h, 0/4h and 4/0 h. Combination Index (CI) is used as a measure of synergism or antagonism. Although all the palladium compounds are less active than cisplatin, the compounds show lower resistance factors. Results show that a number of combinations produce highly sequence- and concentration-dependent synergism. The results of combination will be discussed in terms of cellular accumulation of palladium and palladium-DNA binding levels. If confirmed in *vivo*, the results of the present study may have a profound implication in combination chemotherapy and in devising new strategies to circumvent drug resistance.

Keywords: Ovarian cancer, drug resistance, *trans*-palladiums, phytochemicals, combination.

PO-92a

COPY-NUMBER VARIATION OF THE *UGT2B28* GENE IS STRONGLY ASSOCIATED WITH STROKE COMPLICATION IN BRAZILIAN SICKLE CELL ANEMIA PATIENTS : IMPORTANCE FOR DRUG DISCOVERY AND THERAPY?

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Background - Sickle cell anemia (SCA) is an autosomal recessive blood disorder affecting millions of people worldwide, reducing drastically their life expectancy. In Brazil, the prevalence of this life-threatening disease can reach 1/650. SCA remains difficult to treat notably because of its number of distinct clinical complications such stroke, a leading neurologic cause of death and disability that concerns 24% of SCA patients by the age of 45. Twin and familial aggregation studies performed from the general population, suggest that the risk of stroke has a substantial genetic component. Thus, we postulate that the stroke phenotype observed in SCA patients may partially depend on inter-individual overall sub-microscopic genomic alterations such as single nucleotide polymorphisms (SNPs) and/or copy number variations (CNVs) of > 1 Kb in size.

Objective - To the best of our knowledge, nothing is known about the role of CNVs in the susceptibility to develop a stroke among adult Brazilian SCA patients. So, we undertook a control-case study in order to identify, classify and compare genomic regions where CNVs of a gene would be specifically associated with the risk of stroke.

Materials and Methods - Three independent groups of unrelated adult Brazilian individuals - SCA patients with stroke *aka* "test" (n=18); SCA patients without stroke *aka* "reference 1" (n=16); Healthy individuals *aka* "reference 2" (n=19), were enrolled in this study after approval of the ethics committee FCM-UNICAMP. The patient stratification was performed in accordance to eligibility criteria based on patient's information as well as clinical, laboratorial and imaging data when available. The SCA severity index was estimated for each patient using a specific online calculator [1]. Genomic DNA was isolated from 2 ml of peripheral blood collected from each informed consent patient. CNVs were screened using the Genome-Wide Human SNP Array 6.0 (Affymetrix Inc., CA, USA) and analyzed with the Genotyping Console (GTC) browser software Version 4.1 according to the manufacturer's instructions. Our comparative CNV analysis first focused on large events (> 100 kbps). CNV regions (CNVRs) as well as known candidate genes were identified using the Toronto Database of Genomic Variants (DGV) and NCBI resources. The variants involved in SCA-associated stroke were selected if relative high copy number (CN) frequency was reached. One-Way ANOVA test was elsewhere used.

Results - We found the highest severity score for the "test" (p=0.007) when compared with the "reference 1". No significant differences (p=0.232) were found when comparing the total number of events in the "test" with the

"reference 1" (12.3 ± 4.7 events per patient) or the "reference 2" (12.9 ± 5.9 events per patient). Nevertheless, the number of gains was about three times higher than the number of losses. Among CNVRs of major differences, the chromosome locus 4q13.2 containing the *UGT2B28 gene* was frequently altered by gains in the "test" when compared with the "reference 1" (relative CN frequency=0.039) or the "reference 2" (relative CN frequency=0.05). However, the CN of the candidate gene was rarely altered in the "reference 1" when compared with the "reference 2" (relative CN frequency=0.012), suggesting a specific implication of *UGT2B28* in SCA patients suffering of stroke. The exact size of CNVs varied slightly between patients (119.0 ± 19.6) but, in every case, encompassed the listed gene.

Conclusions - *UGT2B28* gene (encoding uridine diphosphate glucuronosyltransferase (UGT) 2 family, polypeptide B28) was shown to be involved in the human sex steroid metabolism [2] and, more recently, in auto-immunity [3]. The mechanism by which *UGT2B28* CN gene variation is conferred to the susceptibility of developing stroke in SCA patients remains unclear, but may involve steroid inactivation and immune system's ***inflammatory*** response.

Perspectives - Genome wide-association studies (GWAS) are opening new frontiers in the comprehension of diseases and their clinical evolutions. This study in the Brazilian population is getting extended to a larger number of Brazilian samples and, is being validated using other techniques (*e.g.* qPCR) and population groups (*e.g.* HapMap) in order to gain a better understanding about the meaning of such genomic variations. We believe that the clinical outcome of SCA patients can be improved using reliable new genetic markers for diagnosis and therapy.

Keywords: Genome-wide association study; Copy number variations; Sickle cell anemia; Stroke; Brazilian population; Personalized medicine

Financial support – FAPESP

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PO-89

EFFECTS OF SLOW AND SUSTAINED NITRIC OXIDE RELEASING MATERIALS AND ARGINASE INHIBITOR ON HUMAN AORTIC SMOOTH MUSCLE CELL PROLIFERATIONS

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Excessive proliferation of vascular smooth muscle cells (SMC) is primarily responsible for atherosclerosis-a leading cause of death worldwide. Endogenous nitrogen monoxide (NO) or NO provided by NO donors inhibits SMC proliferation. It does so by a combination of effects, reductionof polyamine production and oxidative stress and activation of the NO-cGMP signal pathway. N-nitroso NO donors recently reported by us release NO in a sustained and controlled fashion with tunable rates. The apparent half-lives of these compounds ranged from 18h to 156h. We have prepared 2 new families of NO donors. The NO release profile of each of the NO-donors can be varied by changing the nature of the moieties attached to the N-nitroso group. This is in contrast to clinically approved NO-donors, nitrate compounds, which are known to induce nitrate tolerance. Experimental and commercially available NO-donors exhibit relatively short half-lives. Data obtained from cell culture studies with human aortic smooth muscle cells (HASMC), using one of the N-nitroso NO donors (80 p*M*) exhibited a significant (40%) decrease of SMC proliferation. More importantly, this inhibition was achieved at a very low NO-donor concentration compared to the conventional NO-donors. The physiological effect on HASMC of NO released by these novel NO donors will be discussed.In addition, the effects of a combination arginase inhibitor, ABH, and N-nitroso NO donors to achieve the "right" physiological NO level in cultured HASMC will be presented. Thus our studies will provide further understanding of the effects of NO released in a slow, sustained and rate-tunable manner from the N-nitroso NO donors on HASMC as well as other SMC, cancer and neuronal cells.

PO-43

RETINAL TOXICITY OF INTRAVITREAL TRASTUZUMAB IN A RABBIT MODEL

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Purpose: To evaluate the retinal toxicity of intravitreal trastuzumab in a rabbit model.

Methods: Two groups of six New Zealand albino rabbits each were used. In the first group a concentration of 1mg/0.1ml trastuzumab was injected intravitreally in one eye of each rabbit, while the other eye used as control eye. In the second group 0.1ml of sterile balanced saline was injected into the one eye too. Slit-lamp and funduscopic examinations were performed and the animals were observed for two weeks for signs of inflammation, infection and toxicity. An electroretinogram (ERG) was performed at baseline and 14 days after the injection. The animals were killed on day fourteen and histological examination was performed in the enucleated eyes.

Results: Electroretinogram was greatly affected and in 2 cases extinguished 14 days after trastuzumab injection. Consistent with electrophysiological abnormalities of the retina, signs of retinal edema in experimented eyes, suggesting morphologic retinal damage, were observed. In contrast, in the sham injected eyes, ERG was normal without histopathologic retinal changes.

Conclusion: Intravitreal trastuzumab seems to be toxic to the retina in albino rabbits at a concentration of 1.0mg/0.1ml and could not be safely used as a potential treatment of choroidal or retinal neovascularization.

PO-44

Track: *Drug Discovery in Preclinical Research*

IN VITRO EFFECTS OF VITAMIN SUPPLEMENTS USED FOR AGE-RELATED MACULAR DEGENERATION (AMD) ON PLATELET-ACTIVATING FACTOR (PAF) AND ITS METABOLISM

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Purpose: The purpose of our study was to investigate for the first time a series of vitamin supplements used for Age-Related Macular Degeneration (AMD) as potential inhibitors of Platelet Activating Factor (PAF).

Materials and methods: Various vitamin supplements were tested against PAF-induced platelet aggregation in washed rabbit platelets (WRPs), in order to investigate the interaction between vitamin supplements (InShape, Nutrof, Ocuvite, Vitalux) and PAF or PAF metabolism. Additionally, we examined their ability to affect PAF-metabolism by decreasing PAF activity, through their *in vitro* effect on PAF basic metabolic enzymes (PAF-CPT, lyso PAF-AT, and PAF-AH).

Results: Nutrof exhibited the strongest anti-PAF activity, while Vitalux was the most potent anti-inflammatory factor.

Conclusion: This is the first study to bring in surface potent anti-inflammatory and anti-angiogenic activities of some vitamin supplements used against AMD, through their *in vitro* studied anti-PAF effects in WRPs, suggesting a promising role of resveratrol concerning its potent anti-angiogenic activity in AMD.

PO-96

Track: *CNS Drug Discovery & Therapy*

ANTIFUNGAL ACTIVITY OF FOUR HONEYS OF DIFFERENT TYPES FROM ALGERIA AGAINST PATHOGENIC YEAST : *CANDIDA ALBICANS* AND RHODOTORULA SP.

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Objective: The traditional medicine still plays an important role in the primary health care in Algeria.

Methods: Four Algeria honeys of different botanical origin were analyzed to test antifungal effect against *Candida albicans,* and *Rhodotorula* sp. Different concentrations (Undiluted, 10, 30, 50 and 70 % wt/vol) of honey were studied *in - vitro* for their antifugal activity using *Candida albicans* and Rhodotorula sp.

Results: The range of the diameter of zone of the inhibition of various concentrations of tested honeys was (7-23 mm) for Rhodotorula sp., while *Candida albicans* showed clearly resistance towards all concentrations used. While the MICs of tested honey concentrations against *Candida albicans* and Rhodotorula sp. were (70.09-93.48) and (5.65-99.70) % vol/vol, respectively.

Conclusions: This study demonstrated that, *in vitro*, these natural products had clearly an antifungal activity against Rhodotorula sp and *Candida albicans.*

Keywords: Honey, Antifungal activity, *Candida albicans,* Rhodotorula Sp.

PO-78

Track: *Process Chemistry and Drug Manufacturing*

EFFECT OF THE THERMAL TRAITEMENT ON THE STRUCTURE AND THE ANTACID NEUTRALIZATION CAPACITY OF THE MAGNESIUM HYDROGENOPHOSPHATE TRIHYDRATE

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Antacids are substances able to increase the stomach's pH. Antacids can be formulated as soluble or non-soluble basic salts (NaHCO3 or CaCO3), or basic hydroxides or oxides as Mg(OH)2 or Al(OH)3, or mixed basic hydroxides or oxides of Magnesium and calcium.

The easiest way to decrease gastric acidity is to neutralize it with base because most peptic activity ceases above a pH of 4-5.

In this work, we show that the MgHPO4 ,3H2O neutralization by HCl 0.1N gives an important antacid neutralizing capacity (ANC), in 1 hour at 20°C and the heat treatment of MgHPO4, 3H2O affects its crystalline structure and increases its antacid neutralizing capacity.

A crystalline-amorphous transition of MgHPO4, 3H2O was observed. The X-ray diffraction (XRD), the thermogravimetric analysis (TGA), the differential scanning calorimetric (DSC) and infra-red spectrum (IR) were used in our investigations. The pHmetry was used to determine the ANC of the materials before and after heat treatment.

Keywords: Antacid, Neutralization, Capacity, XRD, TGA, DSC, IR, Amorphous.

PO-82

Track: *Structural Biology*

STRUCTURAL UPDATES OF ALIGNMENT OF PROTEIN DOMAINS AND CONSEQUENCES ON EVOLUTIONARY MODELS OF DOMAIN SUPERFAMILIES

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Early analysis of SCOP database and the statistics of its different versions revealed the presence of overwhelming majority of single-member superfamilies and clearly suggested that the incoming protein structural entries could alter the composition and size of previously accumulated superfamilies greatly. Otherwise, there has been no rigorous analysis of the influence of incoming entries into primary databases, such as protein structural entries on the composition of dependent secondary databases. In this study, we have traced the differences in superfamily compositions (in terms of family constitutents and length variations) between updates of secondary database that relies on SCOP for the definition

of superfamily members, namely PASS2 [versions 2004 and 2008]. Studying length variations, introduced by indels, are important since they have been implicated in introducing substrate specificity, increasing domain interactions and sometimes even regulating protein stabililty. Such an attempt to classify the nature and source of variations in the number and composition of superfamilies during transitions showed that rigidity of the superfamilies had increased in recent version, which may point towards an evolutionary averaging of domains. To study such length variant superfamilies in detail, an improved classification approach is also presented, which divides the superfamilies showing length variations into distinct groups.

Keywords: Length variations, secondary database, protein domain.

PO-103a

Tracks: *Anti-infectives*

TREATMENT OF ACUTE BACTERIAL DIARRHEA IN CHILDREN BY MENTHA PULEGIUM PLANT AS A HERBAL DRUG, A CLINICAL-TRIAL STUDY

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Introduction: Diarrhea is one of the most important global health problems related to children contributing to 3-5 million deaths annually. Mentha Pulegium is traditionally used to treat diarrhea, which is believed to have no harmful side effects. The present study aimed to determine the effect of Mentha Pulegium leaf powder on acute bacterial diarrhea in children.

Method and material: This randomized clinical-trial study was conducted on 70 patients with an age range of 6 months to 5 years with acute bacterial diarrhea. Thirty-five children were treated by ORS. The other thirty-five children, in addition to being treated by ORS, received Mentha Pulegium leaf powder to control their diarrhea. Frequency, volume, and stool consistency at the time of arrival, 24 and 48 hours after arrival were recorded and the results of the two groups were compared. Both the patients and the assessing physicians were not aware of the treatment involved. Frequency distribution tables were used to describe the concentration and dispersion indices. The chi-square test was used for data analysis.

Result and Discussion: The results of the present study indicated that the volume and consistency of stool after the initiation of the treatment, frequency, volume, and stool consistency after 48 hours after treatment showed improvement in the intervention group compared to the control group.

Conclusion: The results of the present study assert the diarrhea treating effect of the Mentha Pulegium plant. The use of this herb is suggested as a non-specific treatment for diarrhea.

Keywords: Diarrhea, Mentha Pulegium, children

PO-63

Track: *Pharmaceutical Research & Development*

SAFETY AND EFFICACY OF DIHYDROARTEMISININ-PIPERAQUINE VERSUS ARTEMETHER-LUMEFANTRINE IN THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN ZAMBIAN CHILDREN

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Background: Malaria in Zambia remains a public health and developmental challenge, affecting mostly children under five and pregnant women. In 2002, the first-line treatment for uncomplicated malaria was changed to artemether-lumefantrine (AL) that has proved to be highly efficacious against multidrug resistant *Plasmodium falciparum*.

Methods: In order to determine whether dihydroartemisinin-piperaquine (DHA/PQP) had similar efficacy, safety and tolerability as AL for the treatment of children with uncomplicated *P. falciparum* malaria in Ndola, Zambia, between 2005-2006, 304 children (6-59 months old) with uncomplicated *P. falciparum* were enrolled, randomized to AL (101) or DHA/PQP (203) and followed up for 42 days.

Results: At day 28, PCR-uncorrected ACPR was 92% in the DHA/PQP and 74% in the AL arm (OR: 4.05; 95%CI: 1.89-8.74; p < 0.001). Day 42 had similar results, i.e. higher PCR-uncorrected ACPR for DHA/PQP, while the PCR-corrected ACPR was similar: DHA/PQP: 93% (179/192), AL: 93% (84/90), (OR: 0.92; 95%CI: 0.30-2.64; p = 0.85). There were no significant differences between treatment arms in the occurrence of adverse events.

Conclusion: DHA/PQP was as efficacious, safe and well tolerated in treatment of uncomplicated malaria as AL, though in the latter group more new infections during the follow up were observed.

Keywords: Malaria, treatment, DHA/PQP, children.

Reference:

Malaria Journal. February 2011; 10:50.

PO-144

EFFECT OF HYDROALCOHOLIC EXTRACT OF *ANETHUM GRAVEOLENS* ON THE DAMAGED PANCREATIC TISSUE IN ALLOXAN MONOHYDRATE-INDUCED DIABETIC RATS TO COMPARE WITH GLIBENCLAMIDE

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Dill with the scientific name of *Anethum graveolens* is a plant from umbellifera. In this research, it was investigated the effect of hydroalcoholic extract of *Anethum graveolens* on the damaged pancreatic tissue in alloxan monohydrate-induced diabetic rats. Also, the effect of extract on changes of serum glucose, lipids and lipoproteins it was investigated and compared to glibenclamide as a chemical drug.

Research method: In order to, twenty adult male rats, weighing 200-250 gr, were used in four groups of five each. Rats in the control group, received physiological serum equal to injecting material volume. Rats in the second group (diabetic), induced diabetes with 120 mg/kgbw dose of alloxan monohydrate. Third group (diabetic + glibenclamide) received 0/5 mg/kgbw glibenclamide after induction of diabetes. Fourth group (diabetic + *Anethum graveolens*) received 300 mg/kgbw dose of *Anethum graveolens* hydroalchoholic extract. Injection of all materials was done via intraperitoneal injection (IP). 48 hours after the last IP, pancreatic tissue comout of each rat body. Then Some sections were made and size of islets, total cells number and the rate of cell proliferation was investigated.

Results: Histomorphological investigation of pancreatic islets showed that the size of pancreatic islets and total number of islet cells is different among the experimental groups. the average of pancreatic islets size and number of islet cells indicated significant reduction in diabetic group compared to control group (P</0 05). State of the treated group with glibenclamide was similar to diabetic group (P>/0 05). Also in pancreatic sections of the treated group with *Anethum graveolens* extract most of the islets have Significantly increased in diameter and they have cell number more than the diabetic and control groups (P</0 05). Also the results from statistical analysis show that *Anethum graveolens* extract reduce the concentration of serum glucose, cholesterol, triglyceride, VLDL-C and LDL-C than diabetic group significanthly (P</0 05). Also, Due to the use of extract, HDL-C level was significantly increased (P</0 05)**.** Effect of *Anethum graveolens* on investigating factors has no significant difference with glibenclamide group (P>/0 05).

Discussion: This research indicate that using of *Anethum graveolens* extract could be effective on regeneration of injured pancreatic islets in diabetic rats. Probably this effect related to antioxidant compounds in exteract. The extract of *Anethum graveolens* increase the rate of cell proliferation. It seems that increase in islet size and cell number is result of remainder cells proliferation in islets. Also the effect of extract in decreasing the concentration of blood suger, serum lipids and lipoproteins than diabetic rats was significant.

Keywords: Diabetes Mellitus, *Anethum graveolens* hydroalcoholic extract, Alloxan monohydrate, Glibenclamide.

PO-11

CAN SEQUENCED COMBINATIONS OF PLATINUMS WITH PHYTOCHEMICALS OVERCOME DRUG RESISTANCE?

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One of the major hurdles in cancer chemotherapy is the development of drug resistance - which is particularly true for the ovarian cancer, where resistant forms often develop. Combination of drugs with different modes of action can offer a means of overcoming chemo-resistance provided the compounds act synergistically in combination. In this study, nine cytotoxic phytochemicals (anethole, betulinic acid, capsaicin, curcumin, genestein, paclitaxel, quercetin, resveratrol and thymoquinone) are applied in binary combination with cisplatin and oxaliplatin to three epithelial ovarian cancer cell lines, A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$ with the aim of providing a means to overcome resistance. The results are analyzed based on the equations derived by Chou and Talalay (1984) with the actual calculations done using Calcusyn software. Generally, bolus combinations are found to be weakly synergistic to antagonistic whereas sequential additions with 2 h gaps are all found to be synergistic, with greater synergism being observed when the phytochemicals are administered first. The degree of synergism is greater in the resistant cell lines than the parent cell line. It is suggested that the combinations of cisplatin and oxaliplatin with the phytochemicals can sensitize the cancer cells to platinum action, thus offering a possible means of overcoming chemo-resistance and opening a new route for cancer treatment.

Keywords: Drug resistance, combination, phytochemicals, platinum drugs, cytotoxicity.

PO-13

Track: *Anticancer Discovery and Therapy*

SYNTHESIS AND *IN VITRO* ANTITUMOR ACTIVITY OF SUBSTITUTED QUINAZOLINE DERIVATIVES: SEARCH FOR ANTICANCER AGENT

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The synthesis of novel 2,3,7-trisubstituted quinazoline derivatives and their biological evaluation as antitumor agents using National Cancer Institute (NCI) disease oriented antit umor screen protocol are investigated. Among the synthesize compounds, twenty compounds were granted NSC code and screened at National Cancer Institute (NCI), USA for anticancer activity at a single high dose (10^{-5}M) in full NCI 60 cell panel. Among the single dose selected compounds, four compounds was found to be the most active candidate of the series at five dose level screening against all panel of cancerous cell line. Rational approach and QSAR techniques enabled the understanding of the pharmacophoric requirement for quinazoline derivatives.

Keywords: Synthesis 2,3,7-trisubstituted quinazoline, Antitumor agents, QSAR, Rational design.

PO-103

ANTI-METASTATIC ACTIVITY OF *CURCUMA XANTHORRIZA* AGAINST HUMAN BREAST CANCER CELLS

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Breast cancer is the most common malignancy and the leading cause of cancer mortality among women worldwide. It is of increasing concern that breast cancer cases diagnosed recently are presenting with chemotherapy-resistant, estrogen independent (ER negative) phenotype with highly invasive metastatic growth properties (1) and metastases, rather than the primary tumours are responsible for most breast cancer mortalities. Interference of tumour metastases is therefore necessary. This has prompted the search for anti-metastatic agents in natural products such as *Curcuma xanthorriza*, member of the Zingiberaceae family. In this study, the methanol, hexane and ethyl acetate extracts of *C. xanthorrhiza* were examined for effects on cellular proliferation, motility and adhesion in the MDA-MB-231, an estrogen independent breast cancer cell line. Treatment of MDA-MB-231 cells with *C. xanthorrhiza* at concentrations ranging from 1µg/ml to 100µg/ml in the MTT assay, wound healing migration assay and cellular adhesion assay showed dose-dependent inhibition of cellular proliferation, motility and adhesion, respectively. Treatment of these extracts on normal lung fibroblast MRC5 cells, has shown to be less responsive, simultaneously suggesting that the extract may be selective towards cancer cells. These findings suggest that the crude extracts of *C. xanthorrhiza* possess the ability to inhibit the

metastasis of breast cancer cells and may therefore be a potential tumour therapeutic agent in suppressing tumour metastases.

Reference:

Gadjos, C., Tartter, P. I., Bleiweiss, I. J., Bodian, C., & Brower, S. T. (2000). Stage 0 to III breast cancer in young women. *Journal of the American College of Surgeons,190*(5), 523-529.

PO-157

A TEN YEAR STUDY OF THE USE OF ANTIRETROVIRAL DRUGS AT THE OBAFEMI AWOLOWO UNIVERSITY TEACHING HOSPITALS COMPLEX, ILE-IFE, NIGERIA

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The prospective and retrospective studies on the use of antiretroviral (ARV) drugs were carried out to investigate pattern of side effects and toxicities, the level of compliance, barriers to compliance, the influence of age, gender, occupation, socio-economic status, educational level and marital status among Nigerians receiving the ARV drugs. The total patients confirmed to have HIV/AIDS as recorded by the Hematology clinic of the Obafemi Awolowo Teaching Hospital Complex (O.A.U.T.H.C), Ile-Ife , Nigeria between July, 1997 and June, 2006 were 1023 patients.

A 37 point structured questionnaire was administered to 571 randomly selected patients with total of 508 respondents. Retrospective study of 123 in-patients of the 508 respondents was carried out with their case notes thoroughly studied. The gender distribution showed that 388(76.4%) were females and 120(23.6%) were males. Marital status showed 473(93.8%)married, 23(4.6%) single and 8(1.6%) widows/widowers. The mean weight was between 56.30kg and 60.50kg.

The results showed that 440 (86.6%) respondents reported no side effects, 20(5.9%) experienced sleep disorders, 12(2.4%) had gastro-intestinal disturbances, 16 (3.1%) had rashes, 5(1.0%) had anaemia and 1(0.2%) had dark spot. Laboratory investigation showed that 35(81.4%) respondents had normal total bilirubin and 8 (18.6%) had value greater than normal. The data was analyzed using student's t-test with Microsoft Version11.0. The study indicated the importance of Pharmacists ability to stress the benefits of ARV therapy during counseling sessions and to enhance the compliance among People Living with HIV/AIDS (PLWHAS).

Keyword: Antiretroviral, Compliance, side effects, Toxicities, Hospitalization.

PO-160

Tracks: *Anti-infectives*

INFLUENCE OF ERADICATION OF HELICOBACTER PYLORI IN PATIENTS WITH DYSPEPSIA REFER TO SHAHEED MOFATEH CLINIC, YASOUJ, IRAN

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To determine whether eradication of Helicobacter pylori (HP) infection is associated with improvement of symptoms severity and satisfaction in comparison with acid suppression therapy in patients with non-ulcer dyspepsia (NUD).

Methods: 100 patients aged 18-45 positive for H.P with nonulcer dyspepsia were randomized into 1 and 2 groups each group 50 patients. The first group was administered a regimen consisting of pantoprazole 40 mg po qd and amoxicillin 1 g and clarithromycine 500mg po bid for 14days. The second group was administered pantoprazole 40 mg po qd, and two placebos for 14 days .all were assigned a follow-up phone call of symptoms improvement after 8 weeks. 13Cbreath tests (13C-UBT) were performed at baseline and during follow-up.

Results: All (100) patients completed the study protocol. In the intention to treat population triple therapy was significantly more effective than acid suppression in complete improvement of dyspeptic symptoms (n=50 , 58% v22%

P = 0.001) . relative improvement of symptom in group 2 compared with triple therapy(G1) is acceptable (n=50 , 56% v34% P = 0.001) .

Conclusions: This study has demonstrated a significant symptomatic improvement at 8 weeks in uninvestigated dyspepsia after eradication of H pylori. Although acid suppression therapy shows an acceptable improvement of symptoms of dyspepsia in HP positive patients and would be an appropriate initial strategy.

Keywords: Rradication, Dyspepsia, *Hlicobacter pylori.*

PO-118

STEP-UP SYNTHESIS OF AMIDOXIME-FUNCTIONALISED PERIODIC MESOPOROUS ORGANOSILICAS WITH AMPHOTERIC LIGAND IN THE FRAMEWORK FOR DRUG DELIVERY

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The step-up synthesis of amidoxime-functionalised periodic mesoporous organosilicas (PMOs) with an ordered hexagonal structure was achieved in two steps: (i) direct co-condensation of diaminomaleonitrile and 3-isocyanatopropyltriethoxysilane (IPTES) and (ii) chemical modification of bridged nitrile into amidoxime using a hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) reagent. The synthesis approach allowed a high loading of amidoxime functional groups in the pore wall framework of the mesoporous materials with controlled regular morphologies. The resulting materials with various diureylenemaleonitrile contents (up to 40 mol %) contained ordered hexagonal mesopores.

High ordered hexagonal arrangement of the pores with a high degree of uniformity of amidoxime-functionalised PMOs was confirmed by low-angle X-ray diffraction (XRD), N_2 adsorption isotherms, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The composition of the mesoporous organosilica was further characterised by Fourier transform infrared (FT-IR) spectroscopy, ^{29}Si magic angle spinning (MAS) and ^{13}C cross polarization (CP) MAS nuclear magnetic resonance (NMR) spectroscopy. The synthesised materials with an amphoteric ligand in the framework were found to be suitable carrier materials for controlled drug delivery systems in a phosphate buffer solution at pH 6.0, 7.4 and 9.0 for both hydrophobic (Ibuprofen) and hydrophilic drugs (5-fluorouracil).

PO-119

CHITOSAN BASED HYBRIDS FOR DRUG DELIVERY

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Chitosan (CS) is a biocompatible, biodegradable, and non-toxic natural polymer and has applications in wound healing, tissue repair, antimicrobial resistance, cell adhesion, and food delivery. In this presentation, we report the facile synthesis of hierarchical mesoporous bio-polymer/silica composite materials with bimodal mesopores using a dual-template of the cationic N,N,N-trimethyl chitosan (TMCs) and the anionic sodium dodecyl sulfate (SDS) via one-step synthetic strategy. The mesoporous bio-polymer/silica composites encapsulate a large number of guest drug molecules, Ibuprofen (IBU) or 5-fluorouracil (5-FU), due to their high surface area and pore volume. In addition, the mesoporous chitosan-silica composites also had a long term biocompatibility for the target release of the drug molecules to the CEM cells and MCF cells etc. as well as a pH sensitive controlled release behavior of the drug molecules. We also present functionalized graphene oxides (GO) with chitosan (FGOCs). FGOCs were found to significantly improve the solubility of the GO in aqueous acidic media. Functionalization chemistry of GO would impart solubility and biocompatibility of FGOCs in biological environment. IBU and 5-FU were loaded on the FGOCs sheets with simple physisorption via π-stacking and inter-atomic interactions. Controlled release behavior of the two drugs was investigated.

PO-34

Track: *Combinatorial Chemistry*

ENANTIOSELECTIVE ADSORPTION AND SEPARATION OF DIPEPTIDES ON A SILICA-GRAFTED CHIRAL CROWN ETHER

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The enantioselective adsorption of several dipeptides on the crown ether-based stationary phase ChiroSil RCA(+) was studied by means of the linear chromatography method. The retention of analytes was measured with acidified water-methanol mobile phases with varied concentration of methanol (from 60 to 90 %, v/v) at different temperatures. Thermodynamic characteristics of adsorption were determined and analysed applying extrathermodynamic relationships. The adsorption isotherms of the LL- and DD-forms of alanyl-alanin were measured to elucidate the adsorption mechanism. A considerable difference in the adsorption of dipeptides with a chiral and achiral N-terminal fragment was proven. An explanation to this fact was proposed assuming that the enantiorecognition of the dipeptides of the first type occurred through the interaction of side groups of the N-terminus with the chiral cavity formed by the crown ether ring. The enantiorecognition of the dipeptides of the second type occurs through the interaction of the C-terminal residue with the side groups of the crown ether moiety.

PO-58

PREPARATION, CHARACTERIZATION AND *IN VITRO* DISSOLUTION STUDY OF NITRAZEPAM: HYDROXYPROPYL- β-CYLCODEXTRIN INCLUSION COMPLEX

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The objectives of this research were to prepare and characterize inclusion complexes of Nitrazepam with hydroxypropyl-β-cyclodextrin and to study the effect of complexation on the dissolution rate of Nitrazepam, a water insoluble drug. The phase solubility profile with hydroxypropyl-β-cyclodextrin was classified as AP-type, indicating the formation of 2:1 stoichiometric inclusion complexes. Gibbs free energy (ΔGtr) values were all negative, indicating the spontaneous nature of Nitrazepam solubilization, and they decreased with increase in the cyclodextrin concentration, demonstrating that the reaction conditions became more favorable as the concentration of cyclodextrins increased. Complexes of Nitrazepam were prepared with cyclodextrin by various methods such as physical mixing, kneading, spray drying & lyophilization. The complexes were characterized by differential scanning calorimetry (DSC), Fourier-transform infrared (FTIR), scanning electron microscopy (SEM) and Powder XRD (P-XRD) studies. These studies indicated complex prepared lyophilization method showed successful inclusion of the Nitrazepam molecule into the cyclodextrin cavity. The complexation resulted in a marked improvement in the solubility and wettability of Nitrazepam. Among all the samples, complex prepared with hydroxypropyl-β-cyclodextrin by lyophilization method showed highest improvement in *in vitro* dissolution rate of Nitrazepam. Mean dissolution time of Nitrazepam decreased significantly after preparation of complexes and physical mixture of Nitrazepam with cyclodextrin. Similarity factor indicated significant difference between the release profiles of Nitrazepam from complexes and physical mixture and from plain Nitrazepam. Tablets containing complexes prepared with cyclodextrins showed significant improvement in the release profile of Nitrazepam as compared to tablet containing Nitrazepam without cyclodextrin. These findings are extremely important from a commercial point of view as the prepared complex removes drawback of poor dissolution profile of Nitrazepam.

PO-17

NANOGEL: A PROMISING TOOL FOR DIABETIC RETINOPATHY

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Purpose: Objective of the project is to formulate noninvasive low cost site specific ocular nanogel drug delivery systems by utilizing natural biopolymers and to target the drug on PKC βII receptor and thereby, reduce the limitations associated with use of intravitreal injection.

Experiments: Curcumin was selected as a prototype for preparation of non-invasive ocular nanogel. The ranges of two polymers (chitosan and ispagol) were selected on the basis of preliminary experimentation. Modified coacervation method was used to produce nanoreservoir system and was optimized by using 2^3 Factorial design. Curcumin nanogels were prepared by carbopol C974 and were evaluated for pH, *in-vitro* gelation study, *in-vitro* ocular irritation test (HET-CAM test) and *in-vitro* permeation study.

Results: Curcumin was docked and found good affinity towards PKC βII receptor. In factorial design the run 8 showed good results with average particle size of 110 nm, zeta potential 38.7 mV, globular in shape (SEM studies) with 84% entrapment efficiency. In case of ocular gels formulation F3 having 1.5% w/w carbopol base showed pH 6.8, consistent and pronounced gel formation, non-irritant followed by more gradual drug release during 24-h period following Korsmeyer-Peppas model (R^2= 0.9942) with non-Fickian diffusion process.

Conclusion: The selected nanogel can be used as a viable alternative to intravitreal injection by virtue of its ability to cross the blood retinal barrier, bind to receptor and sustain the drug release, for its ease of administration and reduced dosing frequency resulting in better patient compliance.

PO-5

Track: *Anti-Cancer Discovery & Therapy*

ENHANCED SYSTEMIC EXPOSURE OF METHOTREXATE IN RATS BY FOLIUM SENNAE AND MECHANISM EXPLORATION

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Folium Sennae (FS, leaves of Cassia angustifolia) is used as a laxative worldwide and a component in commercial products for weight control. Methotrexate (MTX) is a bicarboxylate immunosuppressant with narrow therapeutic window. The pharmacokinetics of MTX was associated with multidrug resistance-associated protein (MRPs) and organic anion transporter (OATs). Judging from the finding that the major molecules in bloodstream after intake of FS are rhein glucuronides/sulfates and rhein, which exist as anions under physiological pH and thus are putative substrates of MRPs and OATs, the metabolites of FS may compete with MTX for these anion transporters. This study investigated the effect of FS on MTX pharmacokinetics. Rats were orally administered MTX with and without FS decoction (FSD). Blood samples were withdrawn at predetermined time points and MTX serum concentrations were assayed by FPIA method. Our results showed that FSD significantly increased the AUC0-2880 and MRT. Cell line study revealed that the metabolites of FSD inhibited the cellular membrane transport of GSMF, an MRP2 probe. In conclusion, FS significantly increased the systemic exposure of MTX through inhibiting the function of MRP 2. We suggest that concurrent use of FS with MTX should be avoided to ensure the safety of MTX.

Keywords: Folium Sennae, methotrexate, herb-drug interaction, multidrug resistance-associated protein.

PO-33

Track: *Hot Topics in Medicinal Chemistry*

EXPERIMENTAL DIABETES TREATED WITH 28-*P*-COUMAROYL-OLEAN-12-ENE-3-O-α-L-ARABINOFURANOSYL-(1→2)-β-D-GLUCOPYRANOSYL ISOLATED FROM *PIPER AURITUM:* EFFECT ON β CELL AND PANCREATIC OXIDATIVE PARAMETERS

Rosa Martha Perez-Gutierrez, Luis B. Flores-Cotera, Carlos Hoyo-Vadillo and Adriana María Neira-Gonzales

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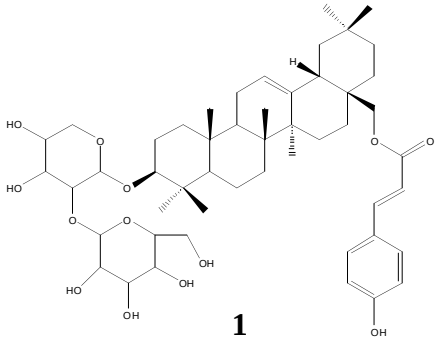
The large-leafed perennial plant *Piper auritum* known as Hoja Santa, is used for its leaves that because of their spicy aromatic scent and flavor have an important presence in Mexican cuisine, and in many regions, this plant is known for its therapeutic properties. We have reported that the methanol extract of *P. auritum* exerted a significant hypoglycemic effect to streptozotocin-diabetic mice. The present study was conducted to investigate the effect of 28-*p*-coumaroyl-olean-12-ene-3-O-α-L-arabinofuranosyl-(1→2)-β-D-glucopyranosyl (**1**) one of the active principles of *Piper auritum*, on the release of insulin secretion and content in pancreatic beta-cells. This compound was identified by spectroscopic analysis. The saponine treatment on streptozotocin-induced type 1 diabetic mice by 28 days on the physiological, metabolic parameters and oxidative stress were determined. Significantly reduced the intake of both food, water and body weight loss as well as levels of blood glucose, serum cholesterol, tryglyceride and increase HDL-cholesterol. The treatment improves pancreatic TBARS–reactive substance level and SOD, CAT, GSH and GPX. The administration of the **1** (10mg/kg) exhibited a significant increase in serum and pancreas tissue insulin. Administration of streptozotocin decreased the insulin secretory activity in comparison with intact mice, but treatment with **1** increased significantly the activity of the beta cells in comparison with the diabetic control rats. The saponin decreased serum glucose in streptozotocin-induced diabetic rats, protect the pancreas-derived β-cells from oxidative stress and increased insulin release from the beta cells of the pancreas in cultured RIN-5F cells. From these results, **1** is suggested to show anti-diabetic effect by stimulating insulin-dependent and by protecting pancreatic β-cells from oxidative stress and also may be an anti-obese, anti-insulin resistance and antihyperglycemic pro-drug.



1

PO-100

Track: *In-silico Drug Design and in-silico screening*

VIRTUAL SCREENING OF MULTI-TARGET INHIBITORS BY COMBINATORIAL SUPPORT VECTOR MACHINES

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Virtual screening (VS) methods have been increasingly explored for searching multi-target agents. In this work, we evaluated the performance of combinatorial support vector machines (C-SVM) in searching dual inhibitors of 29 target pairs from 8 biochemical classes and of different similarity levels between their drug-binding domains. C-SVMs, trained by 68-1894 individual-target inhibitors for the 29 target pairs, were tested on 9-230 dual-target inhibitors collected from literature and produced dual inhibitor yields in the range of 17.65%-77.80% for low similarity target pairs, 14.63%-

73.10% for intermediate similarity target pairs, and 38.26%-75.00% for high similarity target pairs. And the dual inhibitor virtual-hit rates identified by C-SVM in screening 168,000 MDDR compounds were as low as 0.00%-0.28% for low similarity target pairs, 0.02%-0.12% for intermediate similarity target pairs, and 0.03%-0.12% high similarity target pairs. In comparison with the other two VS tools k-Nearest Neighbor (k-NN) and Probabilistic Neural Network (PNN), C-SVM produced comparable dual-inhibitor yields and significantly lower false-hit rates in screening large chemical database, regardless of the similarity level of the target pairs. Combinatorial SVM showed promising capability in searching multi-target inhibitors of target pairs with varying similarity levels.

Keywords: Multi-target inhibitors, high-throughput screening, computer aided drug design, support vector machines, virtual screening.

PO-18

Track: *Drug Delivery & Targeting*

SYNTHESIS OF FE_3O_4@SiO_2@CARBOPOL NANOPARTICLES FOR CONTROLLED DRUG RELEASE

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Multifunctional nanoparticles containing a magnetic Fe_3O_4@SiO_2 sphere and carbopol, a biocompatible polymer, were prepared. Carbopol was grafted to the surface of Fe_3O_4@SiO_2 nanoparticles by amino bonds, and the additional PEG, formed the outermost shell. The anticancer agent doxorubicin (DOX) was loaded into the hybrid nanoparticles via an electrostatic interaction between DOX and carbopol. The release rate of DOX could be adjusted by the pH value using UV-visible spectroscopy.

Keywords: Core-shell nanoparticles, Fe_3O_4@SiO_2 , Carbopol, Controlled drug release.

PO-147

Track: *Cardiovascular Drug Discovery & Therapy*

EPTIFIBATIDE INDUCED SEVERE THROMBOCYTOPENIA IN AN ASYMPTOMATIC PATIENT

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Glycoprotein (GP) IIb/IIIa inhibitors are routinely used in patients with acute coronary syndromes. There have been reported platelet counts of below 20 x 10^9/L within hours of administering the drug. We present a case of a 44 years old man with inferior wall myocardial infarction and third degree heart block who was transferred to our hospital for cardiac catheterization. The patient successfully underwent per-cutaneous intervention to right coronary artery and eptifibatide was given per protocol. 6 hours post-eptifibatide initiation, platelets dropped from 288 x 10^9/L to 24 x 10^9/L. Eptifibatide was stopped and CBC was repeated after 2 hours. The platelets had further dropped to undetectable levels showing 0 x 10^9/L. The patient remained completely asymptomatic. Pseudo-thrombocytopenia was ruled out. Platelet transfusion was considered however, platelets started to climb few hours' post-eptifibatide stoppage. 12 hours later, platelet count reached 4 x 10^9/L. It continued to show a positive trend and reached the baseline of 293 x 10^9/L after 5 days. Patient was discharged in a stable condition. Due to this rare but significant phenomenon, patients on these drugs should have their platelet count closely monitored. It is also very rare not to have any symptoms after such critically low platelet levels.

PO-164

ISOLATION AND CHARACTERIZATION OF ACTIVE THERAPEUTIC COMPONENT FROM AQUEOUS EXTRACT OF TRIDHAM (TD) –A SIDDHA FORMULATION

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Introduction: TD is a combinational Siddha drug traditionally used by Indian Siddha practitioners to combat liver cancer. TD comprises seed coats of *Terminalia chebula*, fruits of *Eleocarpus ganitrus* and leaves of *Prosopis cineraria*.

Hypothesis: TD is proposed to be a potential anticarcinogenic drug, where one of the active component has been isolated and characterized.

Methodology: Total phenol and flavonoid contents, redox potential of TD were analyzed. The effect of TD on HepG2 cell line using trypan blue, MTT, and DNA damage was assessed. Active component was isolated using column chromatography and identified by elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and SEM.

Results: TD aqueous extract was found to exhibit high amount of phenolic and flavonoid contents. DPV showed that TD contains easily oxidizable polyphenols compared to standard and individual ingredients. Systematic *in vitro* studies support the anticancer activity of TD**.** The single crystal X-ray analysis of isolate indicated that gallic acid crystallizes in monoclinic system with space group, P21/c.

Conclusion: TD has been scientifically analyzed for its potential therapeutic agent. This study isolates and evidently characterizes one of the active component gallic acid present in TD.

PO-150

ANTIMICROBIAL AND ANTIPARASITIC ACTIVITIESOF ESSENTIAL OILS FROM *SCHINUS AREIRA* L. (ANACARDIACEAE)

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The *Schinusareira L*. (Anacardiaceae) specie is known by its medicinal value. The essential oils (EOs) of fruits and leaf from *S. areira* L. have been studied for theirantimicrobial and anti–*Trichomonas vaginalis* activities. Fruit and leaf oils of *S. areira*were analyzed separately.

The EOs and the major compound from fruit oil were evaluated for their antimicrobial activities against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella enteriditis*, *Pseudomonas aeruginosa* and *Listeria monocitogenesis*, using broth microdilution method [1].

The EOs from *S. areira* showed antimicrobial activity withMinimal Inhibitory Concentration 12.5to 250 µg/mL. The essential oils from *S. areira* elicited marked *T. vaginilis* inhibition with IC50 between2.5 to 10µg/mL. The essential oil from *S. areira* fruit was more active than that of the leaves in both bioassays.

Reference:

[1] Ericcson, H. M., & Sherris, J. C. (1971). Acta Pathologicaet Microbiologica Scandinavica, 217(1).

PO-153

INHIBITION OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM* BY *LIMONIUMBR ASILIENSE* KUNTZE (PLUMBAGIN ACEAE) EXTRACT

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Infusion from the roots of *Limoniumbrasiliense*Kuntze (Plumbaginaceae) is usedas medicinal plants. The aim of the present study was to evaluate the mutagenic effects in themethanolic extract from the roots of *L. brasiliense.*

The extract was evaluated by *Salmonellatyphimurium* strains TA100, TA98, TA102, TA 1535 and TA 1537, by using AMES test system [1].

The extract showed no biologically relevant increases in revertant colony numbers of any ofthe five tester strains, at concentration of50 – 0.05 mg/mL.

This extract showed antimutagenic activity against sodium azide (NaN3), methyl methane sulphonate (MMS), 2,4,7-trinitro-9-fluorenone (TNF) with percent inhibition of mutagenicity ranging from 61.76% to 86.02% in a concentration-dependent manner.

The methanolic extract from the roots of *L. brasiliense* is not mutagenic, may reduce or inhibit the mutagenic potential of mutagens and carcinogens that conceivably result in cancer as well as diseases caused by genotoxic agents.

Reference:

[1] Maron, D.M. and Ames, B.N. (1983), Mutation Res., 113, 173-215.

PO-16

PERSONALIZED THERAPY OF CLL; THE CHOICE OR NECESSITY

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Chronic lymphocytic leukemia (CLL) is believed to be the commonest adult hematological cancer in the western world. Despite the great improvement in treatment procedures and increase in new anticancer drugs, this type of leukemia remains incurable by cytotoxic therapy.

Selecting the optimal type of treatment for this type of leukemia is not easy for two main reasons. Firstly, the indolent type of disease could last for several years, and when it transforms into an aggressive form, requires immediate therapy. Secondly, individual patients respond differently to the same treatment. Moreover, the coexistence of two populations of leukemic cells, i.e. mainly non-proliferating quiescent CLL cells in peripheral blood, as well as CLL cells with proliferative potential found in proliferative centers of bone marrow or lymph nodes, reflect additional challenge in the search for effective anticancer therapy. Among therapy approaches to this leukemia, much attention is paid to treatment with apoptosis-promoting agent(s).

The aim of study was to determine under *ex vivo* conditions before treatment the chemosensitivity of leukemic cells to combinations of purine analogs (cladribine, fludarabine) with active form of cyclophosphamide - mafosfamide (CM, FM) or rituximab (RCM) to evaluate the apoptosis-inducing potential of the combined treatments.

The biological effects of the exposure of combinations of drugs to CLL cells were determined by several complementary tests. The reduction of the number of living leukemic cells during the course of treatment was analyzed by Vybrant Apoptosis Assay #4. Chromatin changes were monitored by differential scanning calorimetry (DSC) of nuclear fraction isolated from control and exposed to drugs cells. Changes in the expression of selected apoptosis-related proteins were assessed by western blotting.

We have observed a distinct chemosensitivity of PBMCs from the blood of CLL patients to the combinations of drugs used. In the case of CLL cells sensitive to exposure, a significant reduction of the number of living cells was correlated with a marked decrease or even complete loss of thermal transition at 95±3ºC in the DSC profiles of nuclear preparations after treatment. Alterations in expression/proteolysis of apoptosis-related proteins, i.e. strong reduction or loss of Mcl-1 expression, proteolytic cleavage of PARP, caspases activation were also observed. Interestingly, in the cells resistant to the drug combinations examined, no changes in DSC scans and no significant living cell reductions were revealed. Moreover, these data were also confirmed by high expression of Mcl-1 and uncleaved PARP.

The results of our studies confirmed that the development of the personalized medicine based on the individual CLL patient's profiles could increase the rate of complete remission and extend patients' progression-free survival.

PO-98

SYNTHESIS AND EFFECT OF DENDRIMERS ON THE AGGREGATION AND NEUROTOXICITY OF ALZHEIMER'S AMYLOID-BETA PEPTIDE

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Alzheimer's disease (AD) is a major public health concern and there is an urgent need for disease-modifying drugs. AD pathogenesis involves aggregation of the non-toxic amyloid-beta (Aβ) monomer to form soluble highly-toxic oligomers and protofibrils. Inhibition of Aβ aggregation is consequently a promising strategy for the development of disease-modifying drugs. The aim of this project was to develop aggregation inhibitors based on poly-(L-lysine) (PL) and poly(amidoamine) (PAMAM) dendrimers. A series of PL and PAMAM dendrimers with various surface groups were synthesized using solid-phase and solution-phase techniques and the dendrimers were characterized by analytical RP-HPLC, HR-ESMS, and NMR spectroscopy. Thioflavin T (ThT) fluorescence and transmission electron microscopy (TEM) were used to examine the dendrimers influence on Aβ aggregation. A sigmoidal shaped curve was observed for ThT-monitored Aβ aggregation and some dendrimers significantly increased the aggregation lag time and decreased the amplitude. Dendrimers also altered the morphology of Aβ fibrils examined by TEM. The effect of the dendrimers on Aβ-induced cell death was determined via a trypan blue exclusion test of cell viability using cultured mouse primary cortical neurons and neuroprotective dendrimers were identified. This work contributes to an understanding of dendrimer-Aβ interactions and dendrimers for optimization as inhibitors of Aβ aggregation were discovered.

PO-38

5α-REDUCTASE TYPE 1 INHIBITION OF *ORYZA SATIVA* BRAN EXTRACT PREPARED BY SUPERCRITICAL CARBON DIOXIDE FLUID

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The three crude extracts including *Oryza sativa* (bran) from supercritical carbon dioxide fluid ($scCO_2$) process which gave the highest unsaturated fatty acid contents and biological activities including the antioxidative, tyrosinase inhibition, stimulation index on human normal skin fibroblast were selected from ten edible plants to prepare the semi-purified fractions. Fraction No.3 of the *O. sativa* bran crude extract gave the highest content of unsaturated fatty acids and 5α-reductase (type 1) inhibition activity (5AR). Its linoleic acid (LN) and total unsaturated fatty acid (TUC) contents were significantly positive and linear correlated to 5AR on DU-145 cell line (at r of 1.00, p<0.01). Its total phenolic contents and all biological activities also showed positive correlations to 5AR with r > 0.9 (p<0.05). This study has demonstrated the potential of fraction No.3 fractionated from the *O. sativa* bran crude extract prepared by $scCO_2$ to be developed as anti-androgenic alopecia products.

Keywords: Antioxidation, 5α-reductase inhibition, *O. sativa* crude extract, supercritical carbon dioxide ($scCO_2$), unsaturated fatty acids.

PO-143

SUSTAINED IMPROVEMENTS OF FEATURES OF THE METABOLIC SYNDROME UPON NORMALIZATION OF SERUM TESTOSTERONE. FOLLOW-UP UP TO 13.5 YEARS

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Objectives: Hypogonadal men tend to increase body weight, fat mass and develop features of the metabolic syndrome.

Aim: Long-term effects of normalization of testosterone in hypogonadal men on weight, waist circumference and lipid metabolism upon treatment with parenteral testosterone undecanoate.

Design and Methods: A cumulative study of 281 men (134 primary and 88 secondary hypogonadal) (aged 40 ± 13 years), 59 with late onset hypogonadism (LOH). Cut-off point for testosterone treatment was serum testosterone <12 nmol/L. 137 men were studied for at least 4 years and 66 between 5-13.5 years.

Results: A remarkable progressive and sustained decline of body weight and waist circumference, most pronounced over the first 4 years and then maintained. Plasma cholesterol, triglycerides, and LDL-cholesterol showed a similar pattern of decrease. Plasma HDL increased over the first 3 years and maintained in the same order. Systolic and diastolic blood pressure decIined over the first 3 years and maintained.

Conclusion: In both younger (mainly primary and secondary hypogonadism) and older men hypogonadal men (LOH) long-term testosterone treatment improvements of the metabolic syndrome with sustained decline of body weight / waist circumference along with improvements of cholesterol, LDL and triglycerides of similar magnitudes were observed.

PO-142

TESTOSTERONE IS AN EFFICACIOUS AND SAFE TOOL IN THE WEIGHT MANAGEMENT OF ELDERLY HYPOGONADAL MEN

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Background: Obesity negatively affects health and its (drug) treatment is largely unsuccessful. Obesity is associated with low serum testosterone (T), and, conversely, low T leads to weight gain and the metabolic syndrome. This study tested the effects and safety of normalization of testosterone in hypogonadal men.

Materials and methods: 136 hypogonadal men (38 – 83 years, mean 60.6 ± 8.0 years), were treated with parenteral testosterone undecanoate for 4 years as the sole intervention.

Results: A remarkable sustained progressive statistically significant linear decline of body weight, waist circumference, (serum cholesterol, triglycerides, LDL-cholesterol. At baseline 52/136 met the criteria of the metabolic syndrome, after four years 10/136. A slow and steady increase in prostate volume / prostate specific antigen (PSA), PSA never exceeding 4 ng/mL. The residual volume of the bladder and scores on the International Prostate Symptoms Score (IPSS) decreased over the 48 month period. Hemoglobin and hematocrit (Hct) increased significantly reaching their maximum after 12-18 months; at any time point, nine patients had a hematocrit above 52% not requiring interventions.

Conclusion: A significant continuous improvement of features of the metabolic syndrome was noted. Testosterone may be a viable and safe option in weight management.

PO-84

VARDENAFIL ADMINISTRATION PRIOR TO CORONARY OCCLUSION MIMICS ISCHEMIC PRECONDITIONING (IPC) AGAINST INFARCTION AND ARRHYTHMIAS *VIA* CARDIOMYOCYTE MITOCHONDRIAL K_{ATP} CHANNEL ACTIVATION IN INTACT RABBIT HEARTS DURING ISCHEMIA/REPERFUSION

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Objective(s) & Materials and Methods: To test whether the administration of vardenafil(a PDE-5 inhibitor) has cardioprotective and antiarrhythmic effects against ischemia and reperfusion in jury similar to IPC in a well-standardized model of reperfusion arrhythmias in anesthetized adult male rabbits (n=115) subjected to 30 min occlusion of the left coronary artery followed by 120 min of reperfusion.

Results & Discussion: Either one cycle of IPC or vardenafil(0.014 mg/kg, i.v.) prior to coronary occlusion offers significant infarct size reduction (19.7 ± 1.8 % and 18.2 ± 2.0 %, respectively versus 39.2 ± 3.1 % in saline control group; $P < 0.01$) and antiarrhythmic effects. Both IPC and vardenafil treatment significantly attenuated the incidence of

life-threatening arrhythmias like sustained VT (13 % and 25 %, respectively versus 100 % in saline control group; P < 0.005) and other arrhythmias (13 % and 25 %, respectively versus 100 % in saline control group; P < 0.005), and increased the number of surviving animals without arrhythmias. Pretreatment with 5-HD but not HMR 1883 abolished the beneficial effects of IPC and vardenafil on reperfusion induced arrhythmias and cardioprotection indicating that benefits have been achieved via the selective activation of cardiomyocyte mitochondrial K_{ATP} channels. Vardenafil evoked NO release and selective activation of mito K_{ATP} channels leading to IPC in cardiomyocytes contribute to cardioprotection and antiarrhythmic activity during ischemia-reperfusion in the anesthetized rabbit.

Conclusions: We conclude that administration of vardenafil prior to coronary occlusion contribute to IPC-like cardioprotective and antiarrhythmic properties in the intact rabbit heart.

PO-60

CHERRY LAUREL (LAUROCERASUS OFFICINALIS) HAS A POTENTIAL ANTI-DIABETIC ACTIVITY

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Cherry laurel (Laurocerasus officinalis) is used as an anti-diabetic drug in rural co mmunities in the central Anatolia region by the black sea but its biological activity has not yet been scientifically validated. Therefore, we evaluate its anti-diabetic activity.

Forty Wistar-Albino rats weighing 300–350 grams were used in this study. Rats were fed in the standard laboratory living environment for a month. Four groups were studied: healthy, diabetic, glibenclamide and cherry laurel extract. Streptozotocin was injected to induce diabetes. Routine blood glucose, insulin, pre- and intra-anesthesia blood glucose levels were measured and compared. The One-way ANOVA Duncan Test was used for statistical analysis.

At first, control group had better results than other but beginning from the second week, the Cherry laurel extract exhibited superior activity on glucose serum levels to glibenclamide (p is <0.05) and similar with control group. Insulin levels were higher in the extract group, indicating stimulation of insulin secretion. Intra-anesthesia glucose levels in the 600 mg extract group were stable, emphasizing the regulatory function of the extract under stress conditions.

Cherry laurel extract exhibits an anti-diabetic effect, with administration leading to elevated insulin levels, and these preliminary results indicate that further evaluation has to be performed.

PO-101

Track: *Toxicology*

IN VIVO AND *IN VITRO* NEUTRALIZATION EFFECTS OF RAZI INSTITUTE ANTI SNAKE POLYVALENT ANTIVENOM ON INDUCED LOCAL TISSUE DAMAGES BY IRANIAN VIPERA LEBETINA

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Vipera lebetina is a venomous snake found in Africa and much of the middle east including Iran. Myonecrosis, hemorrhage and edema in envenomed patients with this poisonous snake are usually treated by serial injections of Iranian Anti snake antivenom and symptomatic management. Intradermal hemorrhage, paw edema, procoagulant effects and myonecrosis was induced and photographed with injection of multiple doses of our venom to rats in this examination. Our study on this lyophilized venom dissolved in phoshate buffer saline and polyvalent antivenom produced in Iran showed that antivenom was effective and preventive of local tissue damages by incubation with venom prior to injection. Despite myonecrosis and intradermal hemorrhage, local paw edema was not neutralized with premixing with escalating doses of Iranian equine (Fab′)2 antivenom. According to rapid development of poisonous effects in envenomed patients due to metalloenzymes (Gelatinase, Hyaluronidase) and phospholiase A2, Injection of newly introduced neutralizing substances in the field is important in severe envenomations.

Keywords: Vipera lebetina, Iranian anti snake polyvalent antivenom.

PO-148

ANTIMICROBIAL POTENTIAL OF *BALANITES AEGYPTIACA, TYLOPHORA INDICA, STEVIA REBAUDIANA* AND *CASSIA SOPHERA*: ENDANGERED MEDICINAL PLANT SPECIES

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Background: *Balanites aegyptiaca*, *Tylophora indica*, *Stevia rebaudiana* and *Cassia sophera* are declared as endangered plant species in India. Despite their medicinal properties, the antimicrobial potential of these plants has not been explored properly.

Methods: Both aqueous and alcoholic extracts of various parts of respective plants, and their *in vitro* raised calli were tested for antibacterial activity by agar well diffusion method against a range of Gram-positive and Gram-negative bacteria and several fungal species. The calli of *Balanites aegyptiaca* and *Tylophora indica* were only tested as calli of *Stevia rebaudiana* and *Cassia sophera* could not be generated due to failure of regeneration in culture medium. The extracts were also tested against genetically characterized bacterial isolates harbouring *bla* (antibiotics resistance) genes. Minimal inhibitory concentrations (MICs) of the extracts were determined by broth microdilution method.

Results: The extracts from all the four plants showed significant antibacterial activity against Gram-negative bacteria, including, *Salmonella typhi*, *Salmonella paratyphi* A, *Escherichia coli*, *Klebsiella* species, *Proteus vulgaris*, *Citrobacter* spp., *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Vibrio cholerae*. Among all the tested plants, only *B. aegyptiaca* showed significant antibacterial activity against the tested Gram-negative bacteria harbouring *bla* genes including, *Escherichia coli* (*bla* ampC), *Klebsiella* spp. (*bla* CTX-M), *Klebsiella* spp. (*bla* SHV), *Escherichia coli* (*bla* SHV+CTX-M) and *Citrobacter* spp. (*bla* ampC+SHV). Extracts of *B. aegyptiaca* and *S. rebaudiana* also showed activity against Gram-positive bacteria whereas the extracts from *Tylophora indica* and *Cassia sophera* did not. Extracts of *Balanites aegyptiaca*, *Tylophora indica*, and *Stevia rebaudiana* demonstrated potential antifungal activity whereas extracts of *Cassia sophera* din not demonstrate the antifungal activity.

Conclusions: This is the first preliminary report on antimicrobial potential, especially through calli extracts, of these plants and especially against Gram-negative bacteria harbouring *bla* genes.

Key words: *Balanites aegyptiaca*; *Tylophora indica*; *Stevia rebaudiana*; *Cassia sophera*; Antimicrobial activity; *in vitro*; callus; minimal inhibitory concentration.

PO-111

Track: *Stereoselective Synthesis of Bioactive Compounds*

STEREOSELECTIVE MODIFICATION THROUGH *RHIZOPUS STOLONIFER*

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Biotransformation is extensively used for modifications of bioactive compounds accomplishing variety of chemical reactions. Two productds are obtained by stereoselective microbial transformation of dydrogesterone using *Rhizopus stolonifer*. Their structures are confirmed through modern spectroscopic techniques.

Keywords: Biotransformation, dydrogesterone, *Rhizopus stolonifer*

PO-49

Track: *Innovative Drug Discovery and Nanotechnology*

XPS, TEM STUDY Pd, Pd-Ag NANOPARTICLES SUPPORTED ON CARBON SUBSTRATES

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Nanonanoparticles of noble metals are widely used in drug delivery systems and in various areas of biology and medicine. The biological behavior of materials is strongly influenced by the chemical situation on the surface. Pd, Pd-Ag nanoparticles have been synthesized using new methods of pulse laser ablation - deposition (PLA-PLD) on carbon fibers and pyrolytic carbon powder and was characterized by transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS) for their size, morphology, and chemical state. Modifications of charge composition and structure of laser plasma in inhomogeneous electrical, magnetic, electromagnetic fields, without change of parameters of KrF-laser radiation on metal targets allow to change a chemical state of deposited metal nanoparticles and their average size. By results X-ray photoelectron spectroscopy (XPS), at PLA-PLD of Pd (or Pd/Ag ~10:1) on carbon substrates in vacuum (10-3Pa, RT) chemical state palladium can be changed from Pd0 up to Pdn + (1< nanometers). 15 to up 1 from thus modified be can size average their and nanocrystallites are nanoparticles Pd that revealed investigation TEM.

Keywords: XPS, TEM, Pd, Ag, nanoparticles, laser plasma, electric, magnetic fields.

PO-164

***Tracks**: Hot Topics in Natural Products*

SIMULTANEOUS QUANTIFICATION OF MULTIPLE COMPONENTS OF SAN-HUANG-XIE-XIN TANG IN HUMAN PLASMA USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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San-Huang-Xie-Xin Tang (SHXXT) is a widely used Chinese medicine prescription, including Rhei Rhizoma, Coptidis Rhizoma and Scutellariae Radix. The major constituents include aloe-emodin, rhein, emodin, chrysophanol, physcion, coptisine, palmatine, berberine, baicalin, baicalein and wogonin. The metabolism and pharmacokinetics of various constituents of SHXXT in human are mostly unknown. This study aimed to development a rapid and sensitive liquid chromatography-tandem mass spectrometric method to simultaneously quantify the multiple components in human serum and its application to clinical pharmacokinetics. Plasma samples with and without treatment with -glucuronidase/sulfatase were extracted by ethyl acetate and the supernatant was evaporated under N2 gas to dryness before LC/MS/MS analysis. The chromatographic separation of the nine analytes was achieved by a C18 column with gradient elution of a mobile phase containing MeOH and 0.01 % of formic acid. Selected reaction monitoring scanning was performed with switching electrospray ion source polarity between positive and negative modes in a single run. The results showed that the parent forms of coptisine, palmatine and berberine were not detected in plasma. Polyphenols including baicalein, aloe-emodin, wogonin, rhein, emodin, chrysophanol were found predominantly as their sulfates/glucuronides.

Keywords: San-Huang-Xie-Xin Tang, lc/ms/ms, pharmacokinetics

PO-35

***Track:** Toxicology*

IN VITRO NEUTRALIZATION EFFECT OF IRANIAN MULTIVALENT ANTIVENOM ON INDUCED HEMOLYSIS BY *HEMISCORPIUS LEPTURUS* VENOM

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Envenomation by *Hemiscorpius lepturus* is common in south western part of Iran. It is usually accompanied by massive hemoglobinuria, hematuria and in a minority of cases acute renal failure specially in infants. The *In vitro* hemolytic activity of this crude venom on human washed red blood cells was tested. In addition, the temperature and pH stability and neutralizing potency of Razi Institute antivenom - as the standard treatment, was investigated. Complete *In vitro* hemolysis occured with this venom (10µg/ml) in 24 hours. The hemolytic activity dramatically decreased by incubation at 100ºC for one hour showing its proteinaceous structure. In our pH stability test, the venom was abruptly decreased its percent of hemolytic activity (23.4±1.45 at pH=1 versus 98.91±3.41 at pH=9) representing that hemolytic potency of *H. lepturus* was higher in alkaline conditions. Those hemolytic reactions dependent on divalent cations (Calcium and

Magnesium ions) were probably induced by some enzymes like phospholipase(s) present in this venom. Erythrocyte ghost cell formation due to these enzymes(s) by pore forming reaction was induced and photographed in our study. Incubation of erythrocytes with Iranian Antivenom, resulted in sufficient neutralization and stopped the hemolysis, though more investigation is needed for its effectiveness in treating stung patients.

Keywords: *Hemiscorpius lepturus*; Razi Institute multivalent antivenom; metalloproteinase; hemolysis.

PO-28

Track: *Hot Topics in Drug Targets*

SCREENING PROTEASE INHIBITORS IN CYANOBACTERIAL ISOLATES

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Cyanobacteria show compounds with remarkable biological activity, and thus have attracted the attention of the pharmaceutical industry. Proteases are involved in a wide variety of physiological processes in the human body, therefore the search for new protease inhibitors became an issue of great cientific interest. In this work, the distribution of genes coding for aeruginosin and cyanopeptolin (both protease inhibitors), and their characterization by mass spectrometry were investigated. Fragments of *aerA-aerB* and *mcnC-mcnE* gene regions (aeruginosin and cyanopeptolin, respectively) were amplified by PCR and sequenced. Intracellular extracts were screened for protease inhibitors by mass spectrometry. The studied strains showed PCR amplifications to cyanopeptolin and aeruginosin for 43 and 35% from 90 evaluated strains, respectively. Mass spectrometry showed the presence of several aeruginosin and cyanopeptolin variants. To date the production of aeruginosin has been reported for the genera *Microcystis*, *Planktothrix* and *Radiocystis*, however, it was observed amplification for the genera *Synechococcus*, *Geitlerinema*, *Limnothrix*, *Oscillatoria*, *Pseudanabaena*, *Anabaena*, *Aphanizomenon*, *Gloeotrichia*, *Nostoc*, *Fischerella*, *Nodularia*, *Trichormus*, *Sphaerocavum* and *Brasilonema*, while cyanopeptolin was observed for the first time in *Radiocystis*, *Synechococcus*, *Geitlerinema*, *Pseudanabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Fischerella*, *Sphaerocavum* and *Pseudophormidium*. In this study the potential of cyanobacterial strains to produce protease inhibitors led to the discovery of strains with pharmacological properties.

PO-56

Track: *Pharmaceutical Research & Development*

THE EFFECT OF MOLECULAR WEIGHT OF HPMC ON THE SOLUBILITY OF ACETAMINOPHEN FROM SOLID DISPERSIONS

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Introduction: Different methods for increasing solubility of poorly water soluble drugs are in great demand. Forming solid-dispersions, by incorporating the drug into a hydrophilic carrier, is among the most popular methods used for this purpose. Different parameters such as the type and ratio of excipients can affect on the performance of these systems. The aim of the present study was to compare the effect of different molecular weights of HPMC as a water soluble polymer used to prepare solid-dispersion, on the solubility of Acetaminophen (as a poorly water soluble drug).

Material and Methods: Solid-dispersions were prepared by using different molecular weight of HPMC including 15cps and 4000cps, Various ratios of HPMC:Acetaminophen (10:90, 15:85, 20:80 w/w), And solvent evaporation method. The saturation solubility of the prepared samples was studied in distillated water for a period of 24 hours.

Results: The results showed that in all cases solubility was at least 20% higher than the pure drug(12.48mg/ml). HPMC15cps managed to increase the solubility more than HPMC4000cps at all the ratios studied. HPMC15cps at a ratio of 15:85 showed the greatest solubility(17.42mg/ml).

Conclusion: The grade of HPMC used could significantly influence the solubility. At hence should be considered as an important issue in the preparation of solid dispersions of acetaminophen

Keywords: Solid dispersions-Solubility-Acetaminophen-HPMC.

PO-140

SPACE DIFFERENTIATION ANALYSIS ON CHINA PHARMACEUTICAL R&D INNOVATION

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Objective: Positive externality brought about by industrial cluster, has become an important considerations for enterprise site selection. Early detection of technical features in host country can provide valuable references for multinationals R&D investment decision. This study is aimed to analyze current layout of China pharmaceutical R&D innovation and recognize the innovation clusters, for providing necessary reference to investors.

Method: The number of drug patents was selected as proxy variable to measure innovation behavior. Data was collected from China Medicine Patent Database. Quarterback map was performed to range regional innovation and Moran's I index was used to identify the innovation cluster.

Result: 31 provinces in Mainland China were ranged into 4 levels, 8 provinces in the 1st range, with the highest innovation behaviors (accounting to 71.89%), 8 in the 2nd range, and 7 in the 4th range, with the lowest innovation behaviors (only 2.56%). Moran's I index is 0.3645, $p<0.01$.The scatter plot of Local Moran's I index showed High-High and Low-Low differentiation.

Conclusion: Provincial pharmaceutical R&D innovation tended to special cluster in China. Most innovation behaviors occurred in eastern coastal areas. The Yangtze River delta region around Jiangsu is the significant innovation center.

PO-122

AMELIORATING EFFECT OF SELENIUM ON CHROMIUM (VI)-INDUCED OXIDATIVE DAMAGE IN THE BRAIN OF ADULT RATS

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Chromium is known for its wide toxic manifestations. This experiment pertains to evaluate the effect of selenium against oxidative stress induced by chromium in cerebrum and cerebellum. Female Wistar rats were randomly divided into four groups of six each: group I served as controls which received pure drinking water; group II received in drinking water $K_2Cr_2O_7$ alone (700 ppm); group III received both $K_2Cr_2O_7$ and Se (0.5 Na_2SeO_3 mg/kg of diet); group IV received Se (0.5 mg/kg of diet) for 3 weeks. The exposure of rats to $K_2Cr_2O_7$ promoted oxidative stress in cerebrum and cerebellum with an increase in malondialdehyde and a decrease of non enzymatic antioxidants levels such as glutathione, non protein thiol and vitamin C. An increase of enzyme activities like catalase, glutathione peroxidase and superoxide dismutase activities was also observed. Acetylcholinesterase activity was inhibited after treatment with $K_2Cr_2O_7$. Co-administration of Se restored the parameters cited above. The histopathological findings confirmed the biochemical results. In view of the present study, selenium plays the role of an antioxidant which includes free radical scavenging and metal-chelating property and, thereby, improves the detrimental state of brain cells, which unravels its use in the treatment of chromium neurotoxicity and warrants further detailed evaluation.

Keywords: Selenium; Potassium dichromate; Rat; Neurotoxicity; Histopathological studies.

PO-59

VALIDATED STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF ZOLPIDEM IN THE PRESENCE OF ITS DEGRADATION PRODUCTS

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Zolpidem is a hypnotic agent used for the treatment of insomnia. In this study a stability indicating HPLC method was developed for the determination of zolpidem in the presence of its degradation products. Stress degradation of zolpidem was performed under acidic, alkaline, oxidative, heat and photolytic conditions. Separation of zolpidem and its degradation products were performed on a Nova-Pak CN column using KH_2PO_4 30 mM acetonitrile (65: 35, v/v at pH 6) as mobile phase. Acceptable linearity (r^2 >0.999) and precision (CV value <1.5%) was achieved over the concentration range of 1-20 µg/mL.

Degradation of zolpidem was observed under acidic, alkaline, oxidative conditions and also exposure to UV light. The proposed method was used for assay determination of zolpidem tablets with no interfering from excipients.

PO-67

Track: *Translational Medicine*

CHINESE HERB COMPOUNDS INHIBIT HISTONE DEACETYLASE ACTIVITY AND AMELIORATE THE NUROTOXICITY OF MUTANT POLYGLUTAMINE AND TAU IN DROSOPHILA

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All pathogenic mechanisms transcriptional dysfunction has shown to play an important role in the pathogenesis of polygluatmine (PolyQ) mediated neurodegeneration and tauopathy. It is, therefore, rational to believe that modulation of gene transcription activity could be a possible medical intervention for the above neurodegenerations. Indeed, many histone deacetylase inhibitors (HDACIs), compounds that increase acetylation of histones and transcriptional activity, have found to arrest ongoing progressive neuronal degenerations. In the present study, we found that many Chinese herb extracts exhibit strong inhibitory potency for HDAC. Moreover, administration of these herb extracts to Drosophila Spinocerebellar ataxia type 3 (SCA3) and tauopathy models successfully attenuate the cytotoxicity of disease-causing proteins and increase the survivorship of transgenic animals. Currently, the underlying mechanisms by which Chinese herbs mitigate the progression of neurodegeneration are under investigated.

Keywords: Spinocerebellar ataxia type 3, Tauopathy, Drosophila, HDAC inhibitor, Chinese herb compound.

PO-52

Track: *Hot Topics in Natural Prodcut*

RUTIN ATTENUATES CISPLATIN INDUCED RENAL INFLAMMATION AND APOPTOSIS BY REDUCING NFκB, TNF-α AND CASPASE-3 EXPRESSION IN WISTAR RATS

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Cisplatin is an effective chemotherapeutic agent that displays do se-limiting nephrotoxicity. In the present study the wistar rats were subjected to concurrent prophylactic oral treatment of rutin (75 and 150 mg/kg b.wt.) against the nephrotoxicity induced by intraperitoneal administration of cisplatin (7mg/kg b.wt.). Efficacy of rutin against the nephrotoxicity was evaluated in terms of biochemical estimation of antioxidant enzyme activities, histopathological changes and expression levels of molecular markers of inflammation and apoptosis. Rutin pretreatment prevented deteriorative effects induced by cisplatin through a protective mechanism that involved reduction of increased oxidative stress as well as caspase-3, TNF-α and NFκB protein expression levels. We found that the beneficial effect of rutin pretreatment is mediated partially by its inhibitory effect on NFκB and TNF-α pathway mediated inflammation, caspase-3 mediated-tubular cell apoptosis, as well as by restoration of histopathological changes against cisplatin administration.

Keywords: Rutin, Cisplatin, nephrotoxicity, chemoprevention.

PO-132

AMINO ACID SUBSTITUTIONS NEAR ATP BINDING SITE AND PROXIMAL NECK AND THE FAST PACE OF MYOSIN XI

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Cytoplasmic class XI myosins are the fastest processive motors known. This class functions in high-velocity cytoplasmic streaming in plant cells from algae to angiosperms. The velocities at which they process are ten times faster than its closest class V homologues.

To provide sequence determinants and structural rationale for the molecular mechanism of this fast pace of myosin XI, we have compared the sequences from myosin class V and XI through Evolutionary Trace (ET) analysis. We have identified nine myosin XI genes in sorghum and seven in grape by sequence searches. These sequences, along with other plant and animal myosin sequences, were used in ET analysis. The current study identifies class-specific residues of myosin XI spread over the actin binding site, ATP binding site and light chain binding neck region by mapping these class-specific residues on to a crystal structure of homologous myosin V. Further, molecular model of plant myosin XI was obtained. Analysis of putative interactions involving these class-specific residues were performed using the models. This study suggests a structural basis for the molecular mechanism behind high velocity of plant myosin XI. We propose a model of a more flexible switch I region that contributes to faster ADP release leading to high velocity movement of the algal myosin XI.

PO-148

Track: *Protein and Peptide Sciences*

DIFFERENTIAL ACTIONS OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE PAC1 RECEPTORS ON CELL BODIES AND PERIPHERAL TERMINALS OF PRIMARY SENSORY NEURONS

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Pituitary adenylate cyclase activating polypeptide (PACAP1-38) acts at G protein-coupled receptors, the specific PAC1 receptor and VPAC1/VPAC2. PACAP6-38 was descibed as a potent PAC1/VPAC2 antagonist in several models. Maxadilan is a selective PAC1 agonist, its fragment, MAXA65, is a specific antagonist. We aimed at analysing the actions of these peptides on sensory neural responses in vitro.

[Ca2+]i, as the pecific response in cultured rat trigeminal neurones was measured by microfluorimetry. Calcitonin gene-related peptide (CGRP) release from the stimulated peripheral sensory nerve terminals of the isolated rat trachea was measured by radioimmunoassay.

Slowly increasing [Ca2+]i indicating Gq protein-coupled receptor activation was detected both after PACAP1-38 and PACAP6-38 administration, the same response was measured after their co-administration, as well as maxadilan, MAXA65 and their combination (100 nM, 1μM). In contrast, none of these peptides evoked CGRP release from the sensory nerve endings, but they significantly inhibited its electrical field-stimulation-induced outflow at a similar extent.

It is concluded that PAC1 receptor agonism stimulates the cell bodies, but inhibits the stimulation-evoked response of the terminals of primary sensory neurons. Interestingly, peptide fragments acting as antagonists in other models, behave as agonists in these systems. Presently unknown receptors or splice variants might explain these differences.

Keywords: PACAP, PAC1 receptor, sensory neurones.

PO-83

Track: *Hot Topics in Drug Targets*

IDENTIFICATION OF PROMISING THERAPEUTIC TARGETS FROM INFLUENZA GENOMES BASED ON GENETIC, STRUCTURAL, PHYSICOCHEMICAL AND SYSTEMS PROFILES OF SUCCESSFUL TARGETS

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Recent emergence of swine and avian influenza A H1N1 and H5N1 outbreaks and drug resistant strains underscore the urgent need for developing new anti-influenza drugs. Drug development is costly, time-consuming and low in productivity. Limited resources should therefore be focused on anti-influenza drugs against promising targets. Recent studies have shown that promising targets tend to show similar genetic, structural, physicochemical and systems profiles as those of successful targets, and promising targets can be identified by collective analysis of these profiles. We used this approach to identify promising targets from the genomes of influenza A (H1N1, H5N1, H2N2, H3N2, H9N2), B and C. The identified promising targets include neuraminidase of influenza A and B, polymerase of influenza A, B and C, and matrix protein 2 of influenza A. Haemagglutinin of influenza A and B, and hemagglutinin-esterase of influenza C were identified as marginally promising targets. Analysis of the target exploration productivity levels showed fair productivity levels for the identified promising targets, modest levels for the identified marginally promising targets, and low levels for the identified unpromising proteins. Structural studies of the identified promising and marginally promising targets showed druggable and other features that support our identification results.

Keywords: Influenza, Collective analysis, Promising targets, Productivity level.

PO-102

PRODUCTION OF CRUXRHODOPSIN AS A THERAPEUTIC NANOMACROMOLECULE FROM GLYCEROL

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Materials such as functional nanomacromolecules are indispensable in pharmacy and therapy. Nanobiotechnology is a thriving new area of research at the interface between biotechnology and nanotechnology, which deals with structures of dimensions ranging from ~1 nm to ~100 nm. In this study the possibility of cruxrhodopsin (as a nanomacromolecule) production from glycerol was investigated and optimized by Halorcula sp. IRU1. Three important independent parameters (glycerol, yeast extract and KH_2PO_4) were evaluated for their individual and interactive effects on cruxrhodopsin production. The optimum factor levels were a glycerol concentration of 1% (v/v), yeast extract 0.05% (w/v) and KH_2PO_4 0.001% (w/v). The predicted value obtained for cruxrhodopsin under these conditions was about 139.86 mg/l. We can conclude that Haloarcula sp. IRU1 has a high potential for synthesis of cruxrhodopsin from glycerol.

PO-24

OSMOTIC-DRIVEN RELEASE OF PAPAVERINE HYDROCHLORIDE FROM SILICONE IMPLANTABLE ELASTOMERIC MATRICES: A MECHANISTIC STUDY

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Aim of Work: To report on the kinetics of the osmotic-driven release mechanism (ODRM) of Papaverine Hydrochloride (PH) from silicone elastomeric matrices.

Methods: Samples of crosslinked silicone matrices formulated with 10% v/v of PH were prepared and the effects of the device geometry (cylinders, cubes and discs), PH particle size (<63µm, 125-250µm and >250µm) and dissolution media (PBS, distilled water and 3% NaCl solution) on the drug release profiles were studied. Different ratios of elastomer base to curing agent were used to examine the effect of various degrees of crosslinking on the drug release rate. Additionally, the impact of lyophilizing PH with different ratios of trehalose (TH) (1:0.5, 1:1 and 1:2) on the ODRM was studied. The PH released was analyzed using ultra violet spectroscopy and data collected was plotted against time and analyzed.

Results: Devices formulated with the same volumetric loading and smaller drug particles sizes released the drug faster than devices with larger particle sizes. The disk-shaped devices showed faster controlled release profiles than other geometries, with drug release proceeding via typical zero-order release kinetics. The increase in the device's surface area and the incorporation of TH in the loaded lyophilized mix generally increased the PH osmotic release rate. Unexpectedly, it was also noted that samples prepared with higher ratio of curing agent released the drug in a faster rate.

Conclusion: ODRM of water soluble drugs from silicon elastomers depends on the surface area, physicochemical properties of loaded drug and the used elastomer.

Acknowledgements: This work was made possible by a UREP award [UREP 07-120-3-027] to HM Younes from the Qatar National Research Fund (a member of The Qatar Foundation). The statements made herein are solely the responsibility of the authors.

PO-137

A DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF NICKEL (II) AND COPPER (II) USING 6-(ANTHRACEN-2-YL)-2,3-DIHYDRO-1, 2, 4- TRIAZINE-3-TIONE

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Derivative the spectrophotometric method using newly synthesized reagent, 6-(anthracen-2- yl)-2,3-dihydro-1, 2, 4- triazine-3-thione(ADTT), has been developed for the simultaneous determination of copper and nickel. The reagent was synthesized by the acylation of anthracene and then reaction of the resulted product with amylnitrite to produce (anthracen-2-yl)-glyoxal aldoxime. After addition of thiosemicabazone reagent was prepared. Copper and nickel were determined by using zero-crossing method in the second, third and fourth order derivative spectra after derivatization in basic medium.

Beers's law was obeyed in the range of 5-35µg/ml for copper at 387nm (second order) and 554nm (third order) and 5-35µg/ml nickel at 447nm (first order), 400nm (third order) and 385nm (fourth order). The limit of quantification was 5µg/ml for both cations. The within-day and between-day variations in three concentrations were less than 3.05% and 4.73% in all measurements respectively. The proposed method is simple and accurate for determination of binary mixtures.

Keywords: Derivative spectrophotometry, copper, nickel, 6-(anthracen-2-yl)-2,3-dihydro-1, 2, 4- triazine-3-thione.

PO-54

Track: *Drug Delivery & Targeting*

A NOVEL MOLECULAR TARGETED THERAPY FOR THE MUSCULOSKELETAL MRSA INFECTION

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Although Vancomycin (VC) is used for MRSA infection, several issues including nephrotoxicity and insufficient delivery have been reported. It is known that sialyl Lewis X on the leukocytes recognizes its ligand E-selectin in the vascular endothelium, resulting in the leukocytes migration to the extravasucular infection site. Utilizing this mechanism, we have established a novel DDS, in which E-selectin targeted liposome encapsulating VC(VC-Lip) was used. VC-Lip included VC of average 54.5µg/ml and showed sufficient antibacterial effect *in vitro*. The mouse model of gluteus muscle luminescent MRSA infection was used *in vivo*. Daily intravenous administration of VC(VC15mg/kg), VC-Lip(VC22µg/kg, approximately 1/1000 smaller dose than that of VC), or Lip(no VC) were conducted for 10days and the therapeutic effects were evaluated by bio-imaging, serologic and histologic analyses. VC-Lip and Lip signals accumulated to the infection site immediately after administration and MRSA signal in only VC-Lip treated animal was disappeared on 7days. Serologic and pathologic analyses showed the resolution of infection only in VC- Lip treated but not in the VC and Lip treated animals, suggesting that a small amount of VC from target-specific VC-Lip suppressed the infection. This therapeutic strategy can be a safe and effective alternative to standard intravenous administration of VC.

Keywords: Infection, MRSA, drug delivery system, vancomycin, targeting therapy.

PO-3

Track: *Anti-Cancer Discovery & Therapy*

IMPORTANT ROLES OF CELLULAR microRNAs ON LEUKEMOGENESIS BY HUMAN T-CELL LEUKEMIA VIRUS TYPE 1 INFECTION

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A human retrovirus, human T-cell leukemia virus type 1 (HTLV-1) is the causative agent of adult T-cell leukemia (ATL), which is an aggressive and fatal T cell malignancy characterized by dysregulated proliferation of CD4-positive T cells. Transformation of infected cells involves HTLV-1 oncoprotein Tax by modulation of cellular gene expression and/or functions. MicroRNAs (miRNAs), small single strand RNA molecules encoded by the chromosome, regulate gene expression by binding to the target sequence on the 3'-UTR of mRNA, and act as key regulators of many physiological processes involved in carcinogenesis. However, aberrant miRNA expression and its pathologic significance in ATL have not been well documented. In this study, we investigated how miRNAs could be involved in the leukemogenesis by HTLV-1 infection in relation with Tax. Through miRNA microarray and quantitative reverse transcription-PCR analysis, we found that several miRNAs expression levels are altered in HTLV-1-infected T-cell lines comparing to uninfected T-cell lines. To analyze the role of Tax on dysregulation of these miRNAs, we used JPX-9 cells, a Jurkat subclone generated by the stable introduction of a Tax expression plasmid vector. Analysis of miRNA expression in JPX-9 cells showed that Tax induced miR-155 expressions. Previous study demonstrated that miR-155 includes AP-1 and NF-kB transcription factors binding sites, which Tax can activate, on their promoters. Reporter assays demonstrated that miR-155 promoter was induced by Tax through both NF-kB and AP-1 binging sites in its promoter. Electrophoreic mobility shift assay showed that HTLV-1-infected T-cell lines had higher binding activity with AP-1 and NF-kB sites in miR-155 promoter than uninfected T-cell lines. Finally, we found that anti-miR-155 inhibitor reduced the proliferation of HTLV-1-infected T-cell lines but not that of uninfected T-cell lines. In conclusion, our data demonstrate that the expressions of cellular miRNAs are dysregulated in HTLV-1-infected T-cells. HTLV-1 Tax induced miR-155 expression by activating NF-kB and AP-1 signaling pathways. Overexpression of miR-155 enhanced the proliferation of HTLV-1-infected T-cell lines. Our results suggested that microRNAs are good therapeutic targets for treatment of ATL.

PO-8

Track: *Anti-Cancer Discovery & Therapy*

MOLECULAR TARGET FOR TREATMENT OF HUMAN THYROID CARCINOMAS

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Regulator of G protein signaling 4 or RGS4 is a protein which regulates G protein signaling. It belongs to a family of regulatory molecules that act as GTPase activating proteins (GAPs) for G alpha subunits of heterotrimeric G proteins.

We performed a large scale microarray expression analysis of thyroid carcinomas and corresponding normal tissue in Bulgarian patients. A strict purification of carcinoma and normal cells using laser microbeam microdissection was applied. We constructed "genetic map of expression". Several genes of highest expression were analyzed by RT-PCR analysis for validation of their behavior in normal thyroid tissue. RGS4 was selected for functional analysis as a target gene in thyroid carcinoma.

Our results showed elevated expression of RGS4 in human malignant thyroid samples at mRNA level. The results indicate that the RGS4 is thyroid carcinoma specific intracellular protein associated with tumor malignancy. Its inactivation in tumor cell cultures has cytostatic effect and its expression in normal cells stimulates their migration. On the contrary, the gene's expression in healthy thyroid tissue was suppressed. Further on, siRNA experiments on thyroid cancer cell lines reveal the effect of RGS4 overexpression on the growth of cancer cells.

Keywords: Thyroid carcinoma, RGS4, microarray expression analysis, siRNA, functional analysis.

PO-20

IN-VITRO STUDY OF THE EFFECT OF SURFACTANTS ON TRIGLYCERIDE LIPOLYSIS UNDER CONDITIONS MIMICKING THE HUMAN DIGESTIVE TRACT

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Lipid-based drug delivery systems (DDS) improve the bioavailability of hydrophobic drugs. These drugs are usually dissolved or dispersed in lipid droplets, containing mostly triglycerides (TG) and stabilized by surfactants. *In vivo*, these TG droplets must be digested for drug release. Here we study systematically the effects of surfactant type and concentration on the TG lipolysis, with sunflower oil-in-water emulsions. Three types of surfactants were investigated: anionic, nonionic and cationic. The results show that, in absence of bile salts, the TG lipolysis is completely inhibited at high surfactant concentrations, due to formation of dense adsorption layer on the oil-water interface. This layer prevents the enzyme adsorption and direct contact with the TG substrate. In presence of bile salts, we observed three regions in the dependence of TG lipolysis on surfactant-to-bile ratio: in Region 1, the surfactant molecules are solubilized in the bile micelles and the adsorption layer is entirely dominated by bile molecules. In Region 2, mixed surfactant-bile micelles are formed, with high solubilization capacity for the products of triglyceride lipolysis – rapid solubilization of these products leads to complete TG lipolysis for all surfactants studied. In Region 3, the surfactant molecules prevail in the adsorption layer and completely block TG lipolysis.

PO-151

Track: *Pharmaceutical Research & Development*

COMPARATIVE ANTIGENIC PROTEINS AND PROTEOMICS OF PATHOGENIC YERSINIA ENTEROCOLITICA BIOSEROTYPES 1B/O: 8 AND 2/O: 9 CULTURED AT 25^{o}C AND 37 ^{o}C

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Yersinia enterocolitica is a Gram-negative enteric pathogen responsible for a number of gastrointestinal disorders where the most pathogenic bio-serotype is 1B/O: 8. In this study, we compared a pathogenic bio-serotype 2/O: 9 isolated in China to a bio-serotype 1B/O: 8 strain isolated in Japan for antigenicity of the outer membrane proteins and proteomics of the whole-cell proteins. Using two-dimensional gel electrophoresis, we showed the outer membrane protein A (OmpA), C (OmpC), F (OmpF) were the major antigens for both strains; and proteins located on the bacterium's surface and enzymes involved in energy metabolism were also identified as antigenic proteins. We compared the whole-cell proteins of the two strains cultured at 25^{o}C and 37^{o}C and found portions of the outer membrane proteins (OmpX, OmpF and OmpA) were down regulated when the bacteria were cultured at 37^{o}C; whereas some protein subunits: urease subunit gamma (UreA), urease subunit alpha (UreC), urease accessory protein (UreE) involved in urease synthesis were up regulated when the bacteria were grown at 37^{o}C.

PO-107

EFFECTS OF NICOTINE ON PROLIFERATION AND DIFFERENTIATION INTO NEURAL PROGENITOR CELLS IN MOUSE INDUCED PLURIPOTENT STEM CELLS

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Previous studies suggested that Nicotine may play a role in neurogenesis. Induced pluripotent stem (iPS) cells display properties of self-renewal and differentiation into various cells including neural progenitor cells. The present study examined whether Nicotine enhances proliferation or differentiation into neural progenitor cells in mouse iPS cells. BrdU incorporation assay revealed that treatment with Nicotine (300 nM) significantly enhanced proliferation of the cells, which was significantly inhibited by pretreatment with either Mecamylamine (30 μM; an α4 nicotinic acetylcholine receptor (nAchR) antagonist), α-Bungarotoxin (300 nM; an α7 nAchR antagonist), or KN93 (500 nM; a calmodulin kinase II (CaMKII) inhibitor). A fluorescence live cell imaging system using fluo-4 acetoxymethyl showed that Nicotine increased intracellular calcium level dose-dependently. Western blot analysis showed that CaMKII phosphorylation was significantly enhanced by Nicotine treatment. The embryoid bodies derived from mouse iPS cells were transferred to gelatin-coated dishes and then cultured with all trans retinoic acid and Nicotine for 7 days. When the differentiation potential into neural progenitor cells was evaluated by βIII-tubulin expression using western blot analysis, Nicotine did not affect it. These results suggested that the nAchR stimulation may enhance the proliferation of mouse iPS cells via augmentation of intracellular calcium level and CaMKII phosphorylation.

Keywords: Nicotine iPS cells.

PO-136

CHINESE HERBAL MEDICINES DECREASE POLYQ-MEDIATED NEURAL DEATH THROUGH INHIBITION EXCITOTOXICITY

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Polyglutamine (polyQ) expansions in proteins cause several neurodegenerative disorders. Expanded polyQ proteins commonly induce abnormal protein aggregation and apoptosis. Several lines of evidence suggest that excitotoxicity are involved in the pathogenesis of PolyQ-mediated neurodegenerative diseases. Excitotoxicity is the pathological process by which cells are damaged and killed by excessive neuronal excitation owing to excitatory neurotransmitters such as glutamate and similar substances. This occurs when glutamate receptors such as the NMDA receptor and AMPA receptor are over activated. Excitotoxins like NMDA and kainic acid which bind to cell's receptors, as well as pathologically high levels of glutamate, can cause excitotoxicity by allowing high levels of calcium ions (Ca^{2+}) to enter the cell. The Ca^{2+} influx into cells activates a number of enzymes, including phospholipases, endonucleases, and proteases such as calpain. These enzymes go on to damage cell structures such as components of the cytoskeleton, membrane, and DNA. Thus, excitotoxicity is involved in several neurodegenerative disorders such as Alzheimer's disease, Huntington's disease, and spinocerebellar ataxia. To screen novel compounds inhibiting glutamatergic neurotoxicity, the glutamate-mediated excitotoxic cell line of human neuroblastoma SH-SY5Y was used. By using flow cytometry, we found that some Chinese herbal medicines can effectively prevent glutamate-induced cell from death through inhibition of excessive calcium influx. By examining expression of Bcl-2 family protein and other apoptosis-related protein, we found that some Chinese herbal medicines can effectively prevent glutamate-induced cell from death through inhibition of cell apoptosis. In view of polyQ aggregation, we examined whether expanded TBP might induce excitotoxicity. We selected polyQ-mediated cellular models for studying glutamate-mediated associated excitotoxicity with Ca^{2+} influx and cell apoptosis. our preliminary results showed that excitotoxicity contributes to polyQ pathogenesis in SCA17 cell model. Thus, to search novel Chinese herbal medicines targeting glutamate-induced excitotoxicity would be one mechanism for the therapeutic invention.

Keywords: Chinese herbal medicines, polyglutamine, excitotoxicity, apoptosis.

PO-12

THE ROLE OF MOXIFLOXACIN IN THE TREATMENT OF MULTIDRUG-RESISTANT TUBERCULOSIS IN SHANGHAI, CHINA: A RETROSPECTIVE COHORT STUDY

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Background: Moxifloxacin might have additive activity to existing antituberculosis agents. Our aim was to assess the activity of moxifloxacin in the treatment of multidrug-resistant tuberculosis (MDR-TB) in Shanghai, China.

Methods: A retrospective analysis of 203 patients with MDR-TB (from January 2005 to December 2009) receiving with moxifloxacin/levofloxacin-containing regimens was performed. Clinical data was subjected to univariate analysis, stratification, and logistic regression to compare the roles of moxifloxacin with levofloxacin in multidrug regimens.

Finding: Of the 203 patients, 102 patients received moxifloxacin, the remaining 101 patients received levofloxacin together with similar active drugs for similar durations. The overall treatment success rate was 119 of 203 patients (58.6%). Treatment success was predominant in moxifloxacin group compared with levofloxacin group, but did not differ significantly (63.7 vs. 53.4%, $p = 0.14$). Whereas Patients in levofloxacin group had a high treatment failure rate compared with moxifloxacin group, but the difference was also not significant (29.7 vs. 21.6%, $p = 0.18$). The death and default rate, and relapse of patients among two groups was comparative (3.9 vs. 5.9%, $p = 0.51$; 10.8 vs. 9.9%, $p = 0.84$; 15 vs. 13, $p = 0.90$), and one patient in levofloxacin group transferred out during treatment. Cavitary findings, surgical treatment, resistance to ofloxacin, number of agents to which baseline isolate was resistant, and extensively drug-resistant tuberculosis were independent predictors of treatment success.

Interpretation: Moxifloxacin was found to be comparatively efficacious with levofloxacin when incorporated into multidrug regimens used for treatment of MDR-TB in our study.

PO-69

REVERSION OF MULTIDRUG RESISTANCE IN CHEMORESISTANT HUMAN BREAST CANCER CELL LINE BY β-ELEMENE

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Purpose: Multidrug resistance (MDR) presents a serious problem in cancer chemotherapy, and development of agents to overcome MDR is important. The purpose of this study was to investigate the reversal effect of β-elemene on MDR in doxorubicin-resistant human breast carcinoma MCF-7/DOX cells and the parental MCF-7 cells.

Methods: MTT cytotoxicity assays, low cytometry and western blot analysis were performed to investigate the antiproliferative effects of the combination of anticancer drugs with β-elemene, to study the reversal of drug resistance and to examine the inhibitory effects on protein expression.

Results: The results showed that β-elemene (30μM) had strong potency to increase doxorubicin cytotoxicity toward MCF-7/DOX cells with reversal fold (RF) of 6.38. In addition, the mechanisms of β-elemene on reversing P-glycoprotein (P-gp)-mediated MDR demonstrated that β-elemene significantly increased the intracellular accumulations of doxorubicin and Rh123 via inhibiting P-gp transport function in MCF-7/DOX cells. Based on the analysis of P-gp and multidrug resistance-associated protein1 (MRP1) expression using flow cytometry and western blot, the results revealed that β-elemene could regulate down the expression P-gp. However, β-elemene had little inhibitory effect on the expression of MRP1 protein, and the intracellular GSH levels and GST activities in MCF-7/DOX cells.

Conclusions: β-elemene probably represents promising agents for overcoming MDR in cancer therapy.

PO-112

Track: *Regenerative Medicine*

NEUROSURGERY IN COMBINATION WITH TRANSPLANTATION OF OLFACTORY MUCOSA

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The olfactory mucosa and neural stem cells around it also differentiate into local supporting cells that produce growth factors to promote nerve regeneration. Facial nerve decompression surgery is performed to prevent the progression of nerve degeneration, but its effectiveness in patients who have developed nerve degeneration is limited. We devised a method to obtain human nasal mucosa containing nerve stem cells and transplant them to the facial nerve during facial nerve decompression surgery, evaluated growth factor and cytokine production from the nasal mucosa. The facial score was higher with than without nasal mucosa transplantation from 2 to 10 weeks after the onset of paralysis, and the differences were significant 3 and 4 months after the onset.

Healing was accelerated by transplantation of the olfactory mucosa. We cultured structural cells derived from the olfactory mucosa and those from the nasal mucosa of the inferior turbinate. HGF production was more markedly induced by structural cells derived from the olfactory mucosa than by structural cells of the nasal mucosa of the inferior turbinate under stimulation with TNF alpha and dsRNA.

Keywords: Olfactory Mucosa, neurosurgery, facial palsy, stem cell, HGF.

PO-113

Track: *Recent Advances in Patient Treatment and Care*

EFFICACY OF THE CO-ADMINISTRATION OF MONTELUKAST AND LORATADINE FOR SEASONAL ALLERGIC RHINITIS

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Background: Allergic rhinitis to Japanese cedar pollen is peculiar seasonal allergic rhinitis in Japan. An orally administration of the leukotriene receptor antagonist, montelukast, and the H1-receptor antagonist, loratadine, is effective for prophylactic purposes in patients with seasonal allergic rhinitis caused by Japanese cedar pollen (SAR-JP).

Objective: In this study we sought to determine whether the co-administration of montelukast and loratadine was effective for SAR-JP.

Methods: We divided a patient into three groups, montelukast + placebo, loratadine + placebo, montelukast + loratadine. Drug was prophylactic administrated to SAR-JP in the pollen season. We conducted a randomized, double blind, placebo-controlled study to determine the co-administration effects.

Results & Conclusions: The significant difference did not accept it to symptom score between each group, but significantly controlled a sneezing symptom in the co-administration group. In addition, the co-administration group significantly improved total symptom score in cases more than nasal congestion score 2 at past disease severity.

Keywords: Leukotriene receptor antagonist, allergic rhinitis, H1-receptor antagonist, Japanese cedar pollen, co-administration effect, clinical trial randomized double-blind placebo-controlled.

PO-163

THE EFFICACY OF THE COMBINATION OF AZITHROMYCIN AND CLARITHROMYCIN ON MUC5AC PRODUCTION IN AIRWAY EPITHELIAL CELLS

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Background: Airway mucus hypersecretion is an important problem in chronic respiratory diseases. MUC5AC is a major mucin strongly expressed in the lung. The macrolide antibiotics not only possess antibacterial activity, but also exert immunomodulatory effects including reduction of mucin production. We studied the effects of the combination treatment of azithromycin (AZM) and clarithromycin (CAM) on MUC5AC production.

Methods: To induce MUC5AC, NCI-H292 cells were stimulated with 1μg/mL LPS for 24h. To study the effects of macrolides, cells were co-incubated with drugs. MUC5AC protein levels in supernatants were measured by ELISA and MAPKs were evaluated by Western blot.

Results and discussion: MUC5AC was inhibited by treatment with 5μg/mL CAM (217.6 ± 23.3 %, $p < 0.05$ vs. LPS) or 5μg/mL AZM (181.4 ± 46.8 %, $p < 0.01$ vs. LPS) and this inhibition was observed significantly in the combination of CAM and AZM (55.0 ± 17.9 %, $p < 0.01$ vs. CAM or AZM). In the MAPKs study, the level of phospho-ERK was not inhibited by CAM or AZM alone (5μg/mL) but markedly disappeared by the combination of CAM and AZM.

The combination therapy with CAM and AZM may be beneficial to the patients with insufficient by macrolide monotherapy.

PO-30

Track: *Hot Topics in Medicinal Chemistry*

18β-GLYCYRRHETINIC ACID DERIVATIVES INDUCED MITOCHONDRIAL-MEDIATED APOPTOSIS THROUGH REACTIVE OXYGEN SPECIES-MEDIATED p53 ACTIVATION IN NTUB1 CELLS

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Twenty-six 18β-glycyrrhetinic acid (GA) (1) derivatives 2-27 including twelve new GA derivatives 10, 11, 13-17, 21-25 were synthesized and evaluated for cytotoxicities against NTUB1 cells (human bladder cancer cell lines). Seco-Compounds 9, 25, and 27 are the most potent compounds of this series, inhibiting cell growth of human NTUB1 cells. Exposure of NTUB1 to 25 for 24 h significantly increased the production of reactive oxygen species (ROS). Flow cytometric analysis exhibited that treatment of NTUB1 with 25 did not induce cell cycle arrest but accompanied by an increase of apoptotic cell death in a dose-dependant manner after 24 h. Mitochondrial membrane potential (MMP) decreased significantly in a dose-dependant manner when the NTUB1 cells were exposed to 25 for 24 h. Marked collapse of the MMP suggested that dysfunction of the mitochondria may be involved in the oxidative burst and apoptosis induced by 25. Western blot analysis shows that NTUB1 cells treated with 25 increased the level of p-p53 in a dose-dependant manner. Further, NAC treatment prevented p53 phosphorylation stimulated by 25. These results suggested that 25 induced a mitochondrial-mediated apoptosis in NTUB1 cells through activation of p53, which are mainly mediated ROS generated by 25.

Keywords: Synthesis, 18β-Glycyrrhetinic acid, p53, Antioxidant.

PO-31

SYNTHESIS AND ANTI-HCV ACTIVITY EVALUATION OF ANILINOBENZOTHIAZOLE DERIVATIVES

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Hepatitis C virus (HCV) infection is a main cause of chronic liver disease, leading to liver cirrhosis and hepatocellular carcinoma (HCC). Current interferon-based therapy is fraught with severe side effects and has limited cure rate. The objective of our research was to develop effective agents against viral replication. Here, we have synthesized a series of anilinobenzothiazole derivatives. Based on a cell-based HCV replicon system, we observed that compound **10** exhibited anti-HCV activity with a 50% effective concentration (EC_{50}) value of

5 μM. Furthermore, compound **10** showed synergistic anti-HCV activity in combination with interferin-α. We concluded that the compound **10** possessed a potent activity against HCV replication and could provide a promising lead for use in the management of patients with chronic HCV infection.

PO-92

Track: *Drug Metabolism*

ALOE REDUCED CYCLOSPORINE BIOAVAILABILITY IN RATS THROUGH ACTIVATING P-GLYCOPROTEIN AND CYP 3A

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Aloe, the dried leave juice of Aloe barbadensis MILL. or Aloe ferox MILL., is a traditional Chinese medicine and also a popular dietary supplement worldwide. Aloe contains anthraquinone polyphenols including aloin, aloe-emodin and rhein, which have been reported to exhibit beneficial bioactivities. Cyclosporine (CSP), an important immunosuppressant with narrow therapeutic index, is widely used in transplant patients. CSP is a substrate of P-glycoprotein (Pgp) and metabolized by cytochrome P-450 3A4 (CYP3A4). This study investigated the effect of aloe on CSP pharmacokinetics in rats and the relevant mechanisms. Rats were given CSP (Neoral®, 2.5 mg/kg) orally with and without single dose (0.5 g/kg) or seven doses of aloe (0.25 g/kg). Blood CSP concentration was analyzed by a specific monoclonal fluorescence polarization immunoassay. The results indicated that coadministration with single dose or pretreatment with seven doses of aloe significantly decreased Cmax and AUC0-540 of CSP. Furthermore, *in vitro* studies using LS 180 cell line and recombinant isozyme revealed that aloe activated the functions of P-gp and CYP3A4. In conclusion, concurrent use of aloe with CSP markedly decreased the oral bioavailability of CSP through activating P-gp and CYP3A, which may result in allograft rejection in transplant patients.

Keywords: Aloe, Cyclosporine, drug-drug interaction, P-glycoprotein, CYP3A4.

PO-42

Track: *Hot Topics in Natural Products*

INDUCTIVE MODULATION ON P-GLYCOPROTEIN AND CYTOCHROME 3A BY RESVERATROL

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Resveratrol has been reported to show various beneficial pharmacological effects. Nowadays, resveratrol dietary supplement (RDS) is available in the market. Cyclosporin (CsA), a probe drug of P-glycoprotein (P-gp) and cytochrome P450 3A4 (CYP3A4), is an important immunosuppressant. This study investigated the effect of coadministration of RDS on CsA pharmacokinetics.

Rats were orallyadministered CsA alone and coadministered with RDS. The blood samples were assayed for CsA concentrations by FPIA method. Coadministration of 0.1 cap/kg of RDS significantly decreased the AUC0-t and Cmax of CsA by 65% and 72%, and 0.2 cap/kg of RDS reduced those by 78% and 84%, respectively. *In vitro* studies suggested that resveratrol enhanced the activities of P-gp and CYP3A4.

In conclusion, RDS decreased the absorption of CsA through induction modulation on P-gp and CYP3A. Transplant patients treated with CsA should be cautioned against taking RDS to reduce the risk of allograft rejection.

Keywords: Cyclosporine; Resveratrol; P-glycoportein; CYP 3A4.

PO-22

NFκB INHIBITORY EFFECTS OF SALICYLIC ACID DERIVATIVES: IMPLICATION IN N-(5-CHLOROSALICYLOYL)PHENETHYLAMINE AMELIORATES TNBS-INDUCED RAT COLITIS

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To develop a more potent NFκB inhibitor from salicylic acid which is known to inhibit activity of NFκB, a transcription factor regulating genes involved in immunity, inflammation and tumorigenesis, derivatives of salicylic acid (SA) where the 5 position, carboxyl or hydroxyl group was modified were treated in HCT116 cells transfected with an NFκB dependent luciferase gene and LPS-stimulated RAW264.7 cells. Amidation of the carboxylic group or substitution of chlorine at the 5 position increased the ability of SA to suppress the expression of NFκB dependent luciferase and inducible nitric oxide synthase, a product of an NFκB target gene. Moreover, simultaneous amidation and chlorination of SA (5-chlorosalicylamide; 5-CSAM) conferred an additive NFκB inhibitory activity on SA. To further enhance the inhibitory activity, *N*-modification was imposed on 5-CSAM. N-(5-chlorosalicyloyl)phenethylamine (5-CSPA), N-(5-chlorosalicyloyl) 3-phenylpropylamine (5-CSPPA) and N-(5-chlorosalicyloyl) 4-hydroxyphenylethylamine (5-CSHPA) showed greater potencies for inhibiting NFκB activity than other derivatives. Their IC_{50}s' in the luciferase assay measured 15 μM (5-CSPA), 17 μM (5-CSPPA) and 91 μM (5-CSHPA). Rectal administration of 5-CSPA ameliorated TNBS-induced rat colitis, which was more effective than a conventional drug, 5-aminosalicylic acid. These data may provide useful information for development of a therapeutic agent for treatment of diseases where NFκB plays a critical role in the pathogenic progresses.

PO-86

SYNTHESIS AND EVALUATION OF NICOTINIC ACID AND IBUPROFEN CODRUG FOR MANAGING DYSLIPIDEMIA BY A NEWLY DEVELOPED AND VALIDATED HPLC METHOD

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Dyslipidemia is a rapidly and vastly growing disease whose clinical concerns is on the rise especially that it is a predisposing factor for chronic heart disease. Therapeutically, nicotinic acid is the optimum antihyperlipidemic agent and of great clinical interest, yet its intolerable, harmless cutaneous flushing side effect hindered its wide clinical implication. The codrug of nicotinic acid and ibuprofen was synthesized in the aim of resolving the troublesome side effects of nicotinic acid by blockade of prostaglandin synthesis through released ibuprofen; thus, enhance patient's compliance. The synthesized compounds were characterized by 1H-NMR, 13C-NMR, FT-IR, MS, and elemental analysis. Validated HPLC method has been specially developed for the accurate and precise determination of the codrug's physicochemical properties as well as hydrolytic products formed during *in vitro* chemical and enzymatic hydrolysis. The physico-chemical properties of codrug namely solubility, partition coefficient, and pKa were determined. The codrug has a pKa of 3.04 ± 0.20. Its solubility in aqueous and organic solvents was highest in 0.1 M HCl (1.234 mg/mL ± 0.027) and isopropanol (3.905 mg/mL ± 0.057), respectively.

The *in vitro* chemical hydrolysis of codrug and ibuprofen 2-hydroxyethyl ester was evaluated at different pHs and temperatures. It was studied in aqueous phosphate buffer solution of pH 1.2, 6.8, and 7.4 (constant ionic strength) at 70, 80, and 90°C. The hydrolysis was found to be pH- dependent and followed Arrhenius equation. From the Arrhenius plot, the half-life at 25°C and the activation energy in pH 7.4 were extrapolated and were found to be 218 days and 25.01 Kcal mol^{-1}, respectively for codrug, while those of ibuprofen 2-hydroxyethyl ester were 3 years and 22.72 Kcal mol^{-1}, respectively.

The kinetic of enzymatic hydrolysis of codrug and ibuprofen 2-hydroxyethyl ester were evaluated *in vitro* in human plasma and rat liver homogenate. The hydrolysis of codrug and ibuprofen 2-hydroxyethyl ester was faster in human plasma and rat liver homogenate than those in aqueous buffer solutions. The chemical and enzymatic hydrolysis of both, the codrug and ibuprofen 2-hydroxyethyl ester, were found to follow pseudo-first-order kinetics. The pseudo-first-order rate constants were found to be 0.0113, 0.177 $minute^{-1}$ for codrug and 0.0006, 0.0569 minute-1 for ibuprofen 2-hydroxyethyl ester in human plasma and rat liver homogenate respectively. Therefore, this will enable the rapid

liberation of nicotinic acid; managing dyslipidemia, and ibuprofen 2-hydroxyethyl ester, from which ibuprofen is eventually released to alleviate nicotinic acid side effect, cutaneous flushing.

Keywords: Dyslipidemia, Codrug, Nicotinic Acid, Ibuprofen, Validated HPLC, Chemical Hydrolysis, Arrhenius Equation, Enzymatic Hydrolysis.

PO-19

Track: *Drug Delivery & Targeting*

THE INFLUENCE OF FORMULATION AND PROCESS PARAMETERS ON THE MORPHOLOGY, SIZE AND RELEASE PROFILE OF L-DOPA-LOADED POLY LACTIC-CO-GLYCOLIC ACID (PLGA) MICROSPHERES

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Many existing pharmaceuticals are rendered ineffective in the treatment of central nervous system (CNS) diseases due to the highly challenging aspects of drug delivery to the most delicate organ namely brain. In order to overcome the problems of delivering neuroprotective agents to the CNS, numerous strategies have been proposed. Among the developed drug carrier to the CNS, poly lactic-co-glycolic acid (PLGA) microspheres have shown desirable outcomes because of their biocompatibility, biodegradability, convenient processability and resorbability through natural pathways. Meanwhile, a comprehensive understanding of the factors affecting drug release mechanisms from microspheres is critical to the design of optimal drug-loaded microparticles. In the present study, we investigated the physicochemical and emulsifying properties of synthesized L-dopa-loaded microsphere. A series of microspheres of different compositions were prepared by varying the salt (NaCl) content, stabilizer content and homogenizer speed. The prepared microspheres were loaded with L-dopa and characterized by SEM techniques to gain insights into the structural and morphological features. The microspheres size was also determined to elucidate the influence of varying formulation and dynamic properties on the drug release pattern. After evaluating morphology and size of the microspheres, the optimum formulation and process parameters including speed of stirring applied for emulsification, drug concentration, amounts of surfactant and NaCl content in the solvent, were revealed using taguchi software according to the prolonged drug release pattern of microspheres.

Keywords: poly (lactic-co-glycolic) acid, L-dopa, microsphere, NaCl.

PO-90

THE INTERVENTION RESEARCH OF THE EFFECT OF GINKGO BILOBA EXTRACT ON MICE WITH VIRAL MYOCARDITIS

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Objective: To study the interventional effect of Ginkgo biloba extract on mice with viral myocarditis (VMC).

Methods: 160 BALB/C mice were randomly divided into 4 groups: control group, VMC group, VMC+SM group, VMC+GL group. Establish VMC models, at Coxsackie virus B3 (CVB3) infected 5, 10, 15 and 21 days, 8 mice were randomly killed in each group. Then the score for myocardial necrosis and cellular infiltration were examined by HE staining. The level of Protein TGF-β1, Smad2 were detected by immunohistochemical and Western techniques, the correlations were analyzed.

Results: (1) In HE staining VMC group the cardiomyopathy in mice developed with time, inflammatory lesions of various sizes were visible, some were large flakes, in necrotic parts fibrous tissue were visible. The mice with viral myocarditis that received treatment in VMC + SM and VMC + GL group had lighter change compared with VMC group at each time point. (2) The amount of TGF-β1, Smad2 protein expression via immunohistochemical detection of VMC group mice increases significantly over time, positive expression increases significantly compared with control group ($P<0.01$); after intervention on group VMC+SM and VMC+GL, TGF-β1, Smad2 protein expression also reduced among

three groups, P <0.01. (3) The level of relative expression of TGF-β1, Smad2 via Western detection of group VMC mice on each time points increased significantly over time, positive expression significantly increased compared with control group (P<0.05); after intervention on group VMC+SM and VMC+GL, relative level of TGF-β1, Smad2 expression also decreased compared among three groups, P<0.05. (4) The expression of TGF-β1, Smad2 protein of VMC mice myocardial tissue is closely related to pathological points.

Conclusion: TGF β 1-Smad2 signaling pathways may be involved in the process of myocarditis in mice. Ginkgo biloba extract has some therapeutic effect for VMC treatment.

PO-64

TWO RULES ON THE PROTEIN-LIGAND INTERACTION

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So far, we still lack a clear molecular mechanism to explain the protein-ligand interaction on the basis of electronic structure of a protein. By combining the calculation of the full electronic structure of a protein along with its hydrophobic pocket and the perturbation theory, we found out two rules on the protein-ligand interaction. One rule is the interaction only occurs between the lowest unoccupied molecular orbitals (LUMOs) of a protein and the highest occupied molecular orbital (HOMO) of its ligand, not between the HOMOs of a protein and the LUMO of its ligand. Another rule is that only those residues or atoms located both on the LUMOs of a protein and in a surface pocket of a protein are activity residues or activity atoms of the protein and the corresponding pocket is the ligand binding site. These two rules are derived from the characteristics of energy levels of a protein and might be an important criterion of drug design.

PO-172

STUDY ON THE ROLE OF GENERAL PRACTITIONER IN PHARMACEUTICAL ECONOMY OF BOYERAHMAD-IRAN

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Background and objectives: Nowadays one of the largest transactions in the world is pharmaceutical trade. Every day, huge Pharmaceutical companies are trying to manufacture new drugs with better efficacy that lead them to increase great amount of their assets. Doctors that prescribe these products have important role in enhance income of these pharmaceutical industry, so that, the average per capita consumption of drugs is 94 $ worldwide and 21 $ in Iran. Also, Iran is among the twenty high drug consuming countries of the world and ranks second in Asia after China in consuming drug too. According to above, this study was carried out to determine the role of Boyerahmad's general practitioners in treatment cost and pharmaceutical economy.

Methods: In this research, using analysis of prescriptions software, the prescription of Boyerahmad's general practitioners was studied according to mean prices of prescribed drugs .This study has also examined qualitative and quantitative level of correlation between the increment in the prices of drugs and number of items prescribed by each physician.

Results: Finding showed that Boyerahmad's general practitioners divided in 4 following groups based on mean prices of prescribed drugs: group A including 6.45% of physicians that the average cost of their prescriptions was between 10000-19999 Iranian Rial, group B with average cost of 20000-29999 Iranian Rial including 29.04%, group C with average costs of 30000-40000 Iranian Rials(45.16%) and group D with average costs of more than 40000 Iranian Rial included 19.35% of them.

Conclusion: With Respect to results**,** it can be claimed that the frequency of physician in group D with price of prescriptions more than 40000 Iranian Rials, is more than other groups and also,the number of patients who refer to these physicians is more than other too.

PO-128

INVESTIGATION OF ANTIPROLIFERATIVE EFFECT OF KOKUSAGININE AND SKIMMIANINE *IN VITRO*

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Plants and plants extracts play a crucial role in the research of novel antiproliferative agents. More than 60% of the anticancer drugs have a natural origin. The aim of the present study was to determine the cytotoxicity effect of eleven quinoline alkaloids isolated from plants of Rutaceae family. MTT-assay was used to identify the antiproliferative effects of the tested compounds on human adherent cancer cell lines (HeLa, A431, MCF7, A2780). Two alkaloids, kokusaginine and skimmianine were found to inhibit proliferation of cancer cells and to induce cell cycle arrest in dose and exposition time dependent manner in HeLa cells evidenced by flow-cytometry. A non-cancerous human fibroblast cell line (MRC-5) was used to describe the selectivity of the selected agents. The disturbance of the DNA synthesis was confirmed by means of the BrdU incorporation assay. Fluorescent microscopy after Hoechst 33258 – propidium iodide (HOPI) double staining revealed a dose dependent nuclear condensation as well as disturbed cell membrane integrity. Based on the presented results kokusaginine and skimmianine can be regarded as appropriate starting structures for design and synthesis of further quinoline analogs with improved efficacy.

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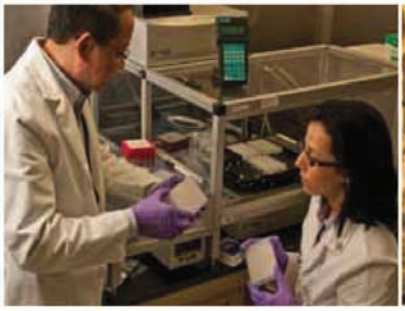
The Found Animals Foundation is a privately funded Los Angeles-based nonprofit organization dedicated to animal welfare issues and led by business and medical professionals. The Found Animals team works directly within the animal welfare community to improve animal shelter processes, fund pet sterilization research, promote effective animal welfare policy, and provide funding to area shelters and spay/neutering clinics. The organization also sponsors and promotes pet adoption, spay/neutering, micro-chipping programs, and various animal and pet-related events.

SRI International


ABOUT SRI'S CENTER FOR ADVANCED DRUG RESEARCH (CADRE)

SRI established the Center for Advanced Drug Research (CADRE) to improve the productivity of the pharmaceutical industry, help our nation respond to biothreats, and develop life-saving treatments for rare and neglected diseases. CADRE's mission is to create new therapeutics, diagnostics, and vaccines for infectious and other diseases and for biodefense. CADRE combines and partners with SRI's well-established expertise in drug discovery, computational biology, and preclinical development with a new proteomics laboratory to develop these solutions.

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SRI's Biosciences division carries out basic research, drug discovery, and drug development, and provides contract (CRO) services. SRI has all of the resources necessary to take R&D from "Idea to IND"®—from initial discovery to the start of human clinical trials—and specializes in cancer, immunology and inflammation, infectious disease, and neuroscience. SRI's product pipeline has yielded marketed drugs, therapeutics currently in clinical trials, and additional programs in earlier stages. In its CRO business, SRI has helped government and commercial clients and partners advance many drugs into patient testing. SRI is also working to create the next generation of technologies in areas such as diagnostics, drug delivery, medical devices, and systems biology.

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Silicon Valley-based SRI International, a nonprofit research and development organization, performs sponsored R&D for governments, businesses, and foundations. SRI brings its innovations to the market- place through technology licensing, new products, and spin-off ventures. SRI is known for world-changing innovations in computing, health and pharmaceuticals, chemistry and materials, sensing, energy, education, national defense, and more.

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Contact for further information

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IMPACT FACTOR: 4.63
(2010 SCI Journal Citation Reports)

Abstracted/Indexed in: BIOBASE/Current Awareness in Biological Sciences, Biochemistry and Biophysics Citation Index®, BIOSIS, Chemical Abstracts, Current Contents/Life Sciences, EMBASE/Excerpta Medica, MEDLINE/Index Medicus, Reference Update, Research Alert, Science Citation Index®, Prous Science Integrity®, Science Citation Index Expanded, PubsHub, Journal Citation Reports/Science Edition, Index to Scientific Reviews®, BIOSIS Previews, BIOSIS Reviews Reports and Meetings, Scopus, EMBASE, BIOBASE, Genamics JournalSeek, J-Gate

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