ABSTRACTS

Multidisciplinary Approaches to Modern Therapeutics:

Joining Forces for a Healthier Tomorrow

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The Bioinformatics of Small Molecules

David Wishart, Professor, Departments of Biological Sciences and Computing Science, University of Alberta, Edmonton, AB, Canada

Small molecules (i.e. those molecules with a molecular weight <1500 Da) are the bricks and mortar of the cell. They serve as the building blocks of the DNA in the nucleus, the proteins in the cytoplasm and the membranes that envelop the cell. They are also the messengers, cofactors, activators and substrates that are responsible for essentially all of the chemistry that goes on inside and outside the cell. Small molecules essentially sit atop an "omics" pyramid built from a multitude of complex genomic, transcriptomic and proteomic interactions. While most of the focus of life scientists over the past 30 years has been on characterizing the large molecules found in our bodies, a few hardy souls have been focusing on using the high throughput technologies originally developed for proteomics or genomics to look at small molecules. This has given rise to the field known as metabolomics – or the study of the small molecule metabolites found in cells, organs or tissues. Metabolomics is an integral part of systems biology and as such, it plays an important role in linking the genome with the proteome and the metabolome. In this presentation, I will introduce the field of metabolomics and show how it relates not only to systems biology but also to the development and discovery of small molecule therapeutics. I will also describe some of our efforts relating to the characterization of the human metabolome (the HMDB) and the development of open access computational resources such as the HMDB, T3DB, DrugBank and FooDB. In particular, I will show how these bioinformatics tools and databases can be used to discover or validate new drugs, new drug targets or new biomarkers of disease.

David Wishart

Dr. David Wishart (PhD Yale, 1991) is a Professor in the Departments of Biological Sciences and Computing Science at the University of Alberta. He is also a senior research officer and the co-director of the Nanobiology program at the NRC's National Institute for Nanotechnology (NINT). He has been with the University of Alberta since 1995 and has published more than 180 articles in various peer reviewed journals. His research interests span many areas including structural biology, bioinformatics, prion biology, nanobiology and metabolomics. From 2006-2009, Dr. Wishart led the "Human Metabolome Project" (HMP), a multi-university, multi-investigator project that catalogued all of the known metabolites in human tissues and biofluids. Using advanced methods in NMR spectroscopy, mass spectrometry, multi-dimensional chromatography and machine learning Dr. Wishart and his colleagues identified or found evidence for more than 8500 endogenous metabolites. This information has been archived on a freely accessible web-resource called the Human Metabolome Database (HMDB). The methods and ideas developed for the HMP have helped lay the foundation for a number of clinical metabolomics projects currently being pursued in his lab. These include studies of several cancer biomarkers, identifying organ transplant biomarkers, and exploring wound healing mechanisms. Dr. Wishart has published more than 40 papers in the field of metabolomics and currently directs the Pan-Alberta Metabolomics Platform – a core facility for metabolomics.

Wednesday AM - Track 1

Progress in Patient-Oriented Research

Patient Oriented Pharmacology Research: Has the Train Left the Station?

Stuart MacLeod, Child & Family Research Institute

The Canadian Institutes for Health Research has introduced a new strategy on patient oriented research with appropriate fanfare. Although not entirely innovative, this initiative coincides with CIHR's continuing evolution towards funding of research likely to improve health outcomes. In this instance opportunities will be created for clinical and evaluative research and the impact is likely to be greatest for those researchers investigating new therapies. CIHR also intends to improve clinical research infrastructure and foster mentoring and training of clinician scientists. While the destination is clear and laudable, the implementation process has been slowed by failure to identify significant new funding or to reallocate resources from older programs.

Success with the new strategy will confirm the importance of clinical and translational research in the modern academic health science network. In particular, Canada's ability to initiate and participate in large multicentre and international trials will be augmented. Key elements in the new strategy include streamlining of ethics review, development of a national template for contracts, and definition of national operating procedures in clinical research, including the development of data management standards leading to a national administrative database. Undoubtedly, strong networks will emerge building on previous Canadian successes in fields such as neonatology, hypertension, oncology, arthritis, medical genetics, cardiovascular science, and maternal and child health. Prospects are already bright for early development of improved networks for mental health and primary care research.

Within the SPOR framework there should be an explosion of methodological development with renewed emphasis on knowledge mobilization and exploitation. CSPT is well positioned to capitalize on these opportunities.

Stuart MacLeod, MD, PhD, FRCPC
Since January 2003 Dr MacLeod has been Vice President Research Coordination & Academic Liaison for the Provincial Health Services Authority of British Columbia and of Pediatrics, Faculty of Medicine, University of British Columbia. Dr MacLeod moved to Vancouver in 2003 to become the Director of the Child & Family Research Institute at British Columbia Children's Hospital and Associate Dean Research at UBC. He stepped down from these roles in January 2010.

Previously Dr MacLeod spent 14 years at the University of Toronto with appointments in the departments of pharmacology, clinical biochemistry, medicine and pediatrics and an adjunct appointment in the Faculty of Pharmacy. He held hospital appointments at the University Health Network (Toronto Western Hospital), The Hospital for Sick Children and the Addiction Research Foundation. At The Hospital for Sick Children, beginning in 1979 Dr MacLeod established the Division of Pediatric Clinical Pharmacology which has become a world leader in research and training of clinician scientists with an interest in pediatric therapeutics and toxicology from a variety of disciplines.

1987 Dr MacLeod became Dean of the Faculty of Health Sciences at McMaster University and in 1992, the founding director of the Father Sean O'Sullivan Research Centre at St Joseph's Healthcare, Hamilton, a position he held until June 2002. During his time in Hamilton Dr MacLeod established a multidisciplinary program in therapeutic evaluation, the Centre for Evaluation of Medicines.

Throughout his career Dr MacLeod has frequently served as an advisor on matters of drug use and investigation to governments in Canada, Ontario, the United States and Europe.

His main research interests at the present time are pediatric clinical pharmacology and toxicology, adverse drug reactions, drug policy and optimal drug use for children. Dr MacLeod has longstanding

involvements in international health that have included work in Africa for international agencies and organizations, including the World Health Organization, Canadian International Development Agency, International Development Research Centre. the Bill & Melinda Gates Foundation and the Rockefeller Foundation. He has worked actively in several countries in eastern, central and southern Africa and, more recently, in Ghana.

Pharmacogenomics of Cardiovascular Drugs

Jean-Claude Tardif, Professor of Medicine, Montreal Heart Institute, University of Montreal

Although no pharmacogenetic test is currently used widely in the treatment of cardiovascular diseases, accumulating data suggest that genotype-based prescribing lies not far ahead, particularly in the case of warfarin. Nonetheless, in recent years, many have highlighted the inconsistencies in results of genomic and pharmacogenomic studies that may transform the "hope" of personalized medicine into an unmet "hype". Hence, minimizing the factors responsible for these inconsistencies will be vital in future studies. More specifically, future studies should be adequately powered. Second, individuals and drug responses should be carefully phenotyped. Third, non-genetic factors such as diet should be assessed and their impact considered. Fourth, in case-control studies, controls should be carefully selected based on detailed demographic characteristics and phenotypic information and not primarily on the availability of DNA samples. Although considerable efforts and progress have been made in recent years to improve technologies which now enable the rapid genotyping of millions of SNPs by high-throughput methods, future efforts should be directed at studying large cohorts of extremely well phenotyped individuals for whom detailed information about non-genetic and environmental factors are collected. Ultimately, these clinical investigations will be responsible for the fulfillment of the promise of individualized medicine to get the right drug, at the right dose, to the right patient at the right time.

Jean-Claude Tardif

Jean-Claude Tardif is the Director of the Research Centre and cardiologist at the Montreal Heart Institute and professor of medicine at the University of Montreal.

Dr. Tardif graduated from the University of Montreal with his medical degree in 1987 and completed his training in cardiology and research in Montreal and Boston in 1994. Dr. Tardif is the Director of the Cardiovascular Health Network of the Quebec Health Research Fund (FRSQ) and also holds the University of Montreal endowed research chair in atherosclerosis. He is the scientific director of the Montreal Heart Institute Coordinating Centre (MHICC) and chairman of the steering committee of the Canadian Atherosclerosis Imaging Network (CAIN) funded by the Canadian Institutes of Health Research

Dr. Tardif has authored and co-authored more than 600 articles and abstracts in peer-reviewed publications including The New England Journal of Medicine, The Journal of the American Medical Association, The Lancet, Circulation, The European Heart Journal and the Journal of the American College of Cardiology. In addition, he has written more than 30 book chapters and has edited several books. He is the principal investigator of several large international clinical trials in the field of atherosclerosis and other cardiovascular diseases.
Because of his accomplishments, Dr Tardif was named Fellow of the Canadian Academy of Health Sciences (FCAHS).

Pharmacogenomics in Children

Evelyne Jacqz-Aigrain, Department of Paediatric Pharmacology and Pharmacogenetics, Clinical Investigation Center Inserm CIC9202, University Paris, Hopital Robert Debré, Paris, France.

The fate and effects of drugs may be different in children and adults and side effects specific to children may occur as children undergo continuous growth and maturation. Children are also different because their diseases are different and some diseases only occur in children. Pharmacogenetic polymorphisms that change the amino acid sequences in coding regions only account for part of the interindividual differences in disease susceptibility and drug response. It is clear since many years that the interactions between developmental patterns of drug metabolising enzymes and transporters have a major impact on dose exposure with age specific dosage requirements. Pharmacogenetic polymorphisms of immunosuppressants, antidepressants, anticancer and/or anti-inflammatory drugs will be presented to illustrate the impact of pharmacogenetic

polymorphisms and age on drug disposition in children. Post-genomic modifications can alter the pattern of gene-expression of the cell through changes in DNA methylation, chromatin and histone modifications, microRNA expression and polymorphisms. Pharmacogenomic approaches, including genomics, proteomics and metabolomics now allow analyzing the environmental influence on drug disposition. These approaches are also involved in the characterization of diseases and in the identification of candidate genes for drug development. Examples will be presented that might explain variability in the regulation of gene expression by genetic and non genetic factors, affecting response to drugs in children. Additional pharmacogenomic and epigenetic studies should be performed to allow individualisation of therapy in children.

Evelyne Jacqz-Aigrain

Is a medical doctor graduated from the University René Descartes in Paris – France. She trained in Paediatrics at the University Hopital Necker-Enfants Malades during a four year residency and was Assistant Professor in Paediatrics and Neonatal Intensive care for two years. She trained as a post-doctoral research fellow in Clinical Pharmacology with Pr C. Dollery in London UK and with J. Oates and G. Wilkinson in Nashville USA and graduated PhD in Pharmacology. She worked for four years as a researcher at INSERM. She is now head of the Department of Paediatric Pharmacology and Pharmacogenetics. – Hopital Robert Debré – Paris – France and responsible of the «French Network of Paediatric Clinical Investigation Centers. She is also an active member of the European Society for Developmental, Perinatal and Paediatric Pharmacology.

Her major areas of interest and activity are in Drug evaluation in children (Immunosuppressants, analgesics, anticancer drugs, drugs in Gastroenterology..) and promotion of safe clinical trials), Pharmacogenetics, Pharmacokinetics in Paediatrics and Neonatology, Population kinetics/dynamics and drug metabolism and ontogeny

Recent Developments in Patient-Oriented Research and Comparative Effectiveness Research

Adrian Levy, Dalhousie University

[Abstract not available]

Wednesday AM - Track 2/3/4

Systems Biology Approaches in Drug Discovery

Looking at Pathways in Cancer in the context of a Large Cancer Genome Sequencing Project

Francis Ouellette, Ontario Institute for Cancer Research, Toronto, ON

Computational biology approaches offer and present a multitude of tools and resources that would allow one to interpret and interrogate what is hidden in the many cancer genomes we have the opportunity to study. In this presentation I will summarize how the OICR executes this, and how we plan to make sense of our data, as well as that from the ICGC as a whole. I will also present some of our recent work on the identification of gene signatures to predict cancer outcomes based on the domain interaction network in human proteome. The results of the quantification in each patient were used to predict cancer outcome by a modified naïve Bayes classifier. In this study, we achieved a favorable accuracy, sensitivity and specificity on a set of well-documented gene expression profiles of breast cancer patients with different outcomes. We also compiled a list of cancer-associated gene signatures and domains, which provided testable hypotheses for further experimental investigation. Our approach proved successful on different independent breast cancer data sets as well as an ovarian cancer data set. This study constitutes a predictive method to classify cancer outcomes based on the relationship between the domain organization and protein network. I will also present how this kind of approach offers new possibilities and directions (and challenges) with data from the ICGC initiative.

Francis Ouellette
Francis Ouellette is a bioinformatician who studies databases, protein networks, and data integration. He is the associate director of the Informatics and Biocomputing platform as well as a Principle Investigator at the Ontario Institute for Cancer Research in Toronto, Ontario. He also coordinates the Canadian Bioinformatics Workshop (CBW). Francis was trained at McGill, Calgary and Simon Fraser University. After working at the Yeast genome project at McGill University and at the NCBI as GenBank Coordinator, he went to UBC in 1998 where he was an Associate Professor in Medical Genetics and Director of the UBC Bioinformatics Centre. He moved to Toronto in August 2007 and is now applying his skills and interest to the study of cancer genomes.

Metabolomics in Translational Medicine and Medicinal Plant Research

W. Jia, G.X. Xie, Department of Nutrition, University of North Carolina at Greensboro, North Carolina Research Campus, Kannapolis, NC.

Various herbal medicines and natural products have the potential to affect a multitude of pathological process, such as carcinogen metabolism, DNA repair, cell proliferation, apoptosis, differentiation, and angiogenesis. Strategies that use a combination of agents of plant origin, rather than individual phytochemical compounds, offer a potential for maximizing disease prevention while minimizing toxicity. This principle, which has been practiced for hundreds of years in traditional medicines such as traditional Chinese medicine, and is routinely applied to therapeutic treatments of cancer in Western medicine, is fully applicable to disease-prevention by bioactive food components.

The pharmacology of multi-component agents, including botanical-based nutraceuticals, entails a “network” approach, in which multiple compounds interact with multiple targets *in vivo* with interdependent activities to achieve an optimal effect. Simultaneous evaluation of pharmacokinetics (PK) and pharmacodynamics (PD) for multicomponent herbal medicines and natural products is difficult due to the vast number of compounds present in natural products, their wide

range of concentration levels, and the complexity of their interactions and degradations *in vivo*. We developed an integrated profiling approach coupled with multivariate statistical analysis that simultaneously characterized PK and PD of a dietary intervention with a Chinese tea (Pu-erh) in human subjects. The dynamic concentration profile of bioavailable plant molecules due to *in vivo* absorption and the hepatic and gut bacterial metabolism, as well as the human metabolic response profile were quantitatively measured within a single experiment. This novel approach will address the bottleneck problem in the pharmacological evaluation of multi-component botanical agents, leading to a direct elucidation of systems pharmacology and molecular mechanisms of the herbal medicines and natural products.

Wei Jia

Dr. Jia's M.S. and Ph.D. were completed at the University of Missouri-Columbia in the field of radiopharmaceutical chemistry. Dr. Wei Jia, has worked nearly a decade on biochemical profiling and evaluation of botanical preparations (traditional Chinese medicine, TCM) in pharmacological models. He pioneered the application of metabolomics approaches to TCM research and has taken leading roles in several important studies funded by Chinese Ministry of Science and Technology and Shanghai Science and Technology Commission. He is the author of over 130 scientific papers and 4 books, and the speaker of over 45 invited lectures and talks at major life sciences and pharmaceutical-based conferences and institutes. He serves on the editorial boards for 10 scientific journals of -omics sciences, TCM and herbal medicines, and an expert reviewer for State Food and Drug Administration of China, Chinese Ministry of Science and Technology, Natural Science Foundation of China, and Research Grants Council of Hong Kong.

Professor Jia's current research focuses on mass spectrometry (MS)-based metabolomics profiling technologies, metabolic phenotypes and metabolic transformation in cancer, and pharmacokinetic and metabolomic characterization of TCM and natural products. He utilizes global metabolic and chemical profiling approaches, as a way to scientifically bridge different pharmaceutical and nutritional concepts and methodologies. A top-down or 'from whole to parts' strategy is taken in his research to capture the holistic and dynamic variations of biological systems in response to environmental stimuli or drug intervention, and to elucidate the underlying mechanisms of disease onset and pathological development.

Dr. Jia was previously Professor of Natural Medicines and Vice Dean, School of Pharmacy, Shanghai Jiao Tong University. Dr. Jia was also the Principal Investigator and Leader of the Metabolomics Program at Shanghai Center for Systems Biomedicine. This organization conducts innovative and cross-disciplinary research creating novel solutions to clinical problems.

Systems Biology Analysis of Kinase Inhibitors in Cancer Therapy

Jacques Colinge, Center for Molecular Medicine of the Austrian Academy of Science, Vienna, Austria

Chemical proteomics provides methods to measure drug-protein physical interactions in specific cell types in an unbiased manner. It is especially interesting for compound classes such as kinase inhibitors that can be rather unspecific, targeting tens of kinases, several of them with high affinity. It is therefore important to analyze such target profiles at a system level, taking into account possible synergies of the targeted proteins.

We present computational methods to relate a drug to its potential systemic effects through the knowledge of its protein targets and their mapping onto the human interactome, i.e. the known protein-protein interactions. Considering the global topology of the interactome as well as protein functional annotations, it is possible to identify a region of "drug influence" within the interactome and to use it to predict the action of the compound as well as its potential side-effects.

Similar approaches can be applied to estimate the influence of disease-causing genes or proteins to the interactome, as a model of the cell biology. Combining the two aspects, drug and disease influences, it is possible to compare different drugs and to predict their relative efficacy as well as some potential additional applications.

The global approaches we present are generally applicable and they fit kinase inhibitor specifics well. As a matter of fact, these compounds cause difficulties in the systems biology analysis because they often targets heavily connected proteins, i.e. hubs. Such hubs easily "dilute" the information during the functional analysis if a global topology perspective is not adopted.

Jacques Colinge

Dr. Jacques Colinge (PhD and M.S. in mathematics, University of Geneva, Switzerland) is Head of Bioinformatics at Ce-M-M in Vienna (http://www.cemm.oeaw.ac.at) since 2006 and an adjunct professor (Habilitation) at Graz Technical University in Austria since 2009.

His current scientific interests cover various aspects of proteomics from statistical modeling of mass spectrometry data to the systems biology analysis of protein interaction networks. Current research focuses on quantitative proteomics of posttranslationally modified proteins, prediction of protein complexes, drug action modeling, and the analysis of protein interaction networks. Previously, Dr. Colinge worked on differential gene expression and the numerical analysis of strongly non linear partial differential equations.

Previous, Dr. Colinge has served as a Professor of Bioinformatics at the Upper Austria University of Applied Sciences in Hagenberg (2005-2006), mass spectrometry bioinformatics and parallel computing group leader at GeneProt Inc. (2000-2005), bioinformatician at Serono Pharmaceutical Research Institute (1998-2000), Research Assistant at University of Geneva Mathematics Department (1993-1998). Before, Dr. Colinge obtained a degree in computer science for business applications and worked as freelance software developer and computer programming teacher.

Wednesday PM - Track 1

Erythropoietin and Cardiovascular Risk

Effect of Erythropoietin on Blood Pressure and Blood Vessels

Marcel Lebel, CHUQ Research Center, L'Hôtel-Dieu de Québec and Department of Medicine, l'Université Laval, Quebec City

Erythropoietin (EPO) acts on many non-erythropoietic tissues and its receptor (EPOR) is expressed in several cell types including endothelial cells, neurons and cardiomyocytes. In acute conditions such as ischemia/reperfusion, EPO at high doses exerts protective effects. On contrary, during chronic EPO replacement therapy (low doses) for anemia in chronic kidney disease (CKD) patients, EPO can cause *de novo* hypertension or exacerbate pre-existing hypertension. This adverse effect on blood pressure is seen almost exclusively in CKD; indeed, EPO does not increase blood pressure in healthy subjects. The precise mechanism underlying the development of hypertension following EPO therapy in CKD is still unclear. This pressor effect cannot be accounted for by an increase in hematocrit and blood viscosity since anemia in these patients is never completely corrected. Other potential mechanisms for the inappropriate increase in peripheral resistance during EPO therapy include enhanced tissue renin activity, increase in angiotensin II receptor expression, nitric oxide resistance and increased oxidative stress. We recently reported that EPO also stimulates the vascular production of endothelin-1 (ET-1) and accentuates the pre-existing endothelial dysfunction in CKD. The mechanisms of this iatrogenic form of hypertension in CKD patients will be discussed.

Marcel Lebel
Dr. Marcel Lebel is a Professor of Medicine with Laval University's Department of Medicine and a Nephrologist, specialized in hypertension at the CHUQ Research Center of L'Hôtel-Dieu de Québec Hospital. Dr. Lebel is a graduate of Laval University where he completed his training in both Internal Medicine and Nephrology. Under a Medical Research Council Fellowship, he completed his research training at the Clinical Research Institute of Montreal and at the MRC Blood Pressure Unit in Glasgow, Scotland. Thereafter, he was a Scholar of the Fonds de la recherché en santé du Québec (FRSQ).Dr. Lebel's ongoing research interests include both the role of the kidney and of vasoactive hormones in the pathogenesis of primary and secondary hypertension. His peer-reviewed research is supported by operating grants from the Canadian Institute of Health Research. He recently received the "Distinguish Service Award" from the Canadian Hypertension Society, the "Founder's Award" from the Kidney Foundation of Canada and the 2010 Scientific Mentor Award of the CRCQ. Dr. Lebel is a Past President of the Canadian Hypertension Society, the Canadian Society of Nephrology and the Quebec Hypertension Society. He is a renowned clinical scientist who, throughout his career, has made a significant contribution to the field of hypertension and Nephrology.

Effects of Erythropoietin in Myocardial Infarction and Potential Risks of Thrombosis

Anargyros Xenocostas, University of Western Ontario

Although erythropoietin (EPO) is a molecule that primarily drives erythropoiesis in the bone marrow by acting as a survival factor, it also exerts multiple pleiotropic effects on a variety of different cells and tissues including those of the cardiovascular system.

Over the past decade tissue-protective effects of EPO have been described in various injury models including reversible and irreversible myocardial ischemia, where decreases in infarct size, prevention of deleterious remodeling, improved cardiac function and survival have been noted. Tissue protection appears to be mediated by multiple

mechanisms/pathways including inhibition of apoptosis, anti-inflammatory effects, mobilization of endothelial cell precursors with promotion of angiogenesis and anti-arrythmogenic effects.

However, the future application of EPO for cardioprotection may be limited by its additional effects on the coagulation system and circulation. Treatment of renal patients has been associated with hypertension and increasing hemoglobin levels to near normal are associated with increased risk of cardiovascular events and death. Furthermore, EPO therapy can lead to platelet activation and risk of venous occlusion where the risk of thrombosis is well described in the cancer patients and is a potential concern in patients undergoing revascularization or stenting procedures. Other effects on the coagulation system remain to be defined. These issues may be overcome by non-erythropoietic analogues of EPO that are protective but are not platelet activating.

Nonetheless, the preclinical data strongly support a tissue protective role for EPO in the CVS and a number of ongoing clinical trials will soon be able to determine efficacy in the clinical setting.

Erythropoietin Stimulating Agents in Chronic Kidney Disease

Brendan Barrett, Memorial University, Newfoundland

In 1990 the Canadian Erythropoietin Study Group demonstrated that the partial treatment of severe anemia with Eprex in hemodialysis patients substantially reduced the use of blood transfusions and significantly improved quality of life. However the safety of this intervention compared to placebo has never been studied. The efficacy of treatment of moderate anemia (baseline Hemoglobin >10g/dl) with ESAs in both pre-dialysis and dialysis patients has been examined in 4 randomized controlled trials ,where normalization of hemoglobin was compared to partial correction of anemia, and in one large trial compared to placebo. A hemoglobin target> 13g/dl was associated with harm—increased incidence of strokes ,hypertension ,and vascular access clotting--,and there was no improvement in cardiac structure and function or in cardiac event rates. A signal that cancer outcomes may be worse compared to partial correction of anemia was reported in TREAT. Furthermore quality of life benefits were inconsistent across trials and the transfusion benefit was modest. The following recommendation for ESA use seem reasonable. (1) If Hb falls below 9 g/dl start ESA to prevent transfusions.(2) if Hb is between 9.0 and 10. individualize therapy based on stage of CKD and presence of symptoms.(3) Target Hb 10.0-11.5 or 12.0 .(4) avoid Hb levels >13.0.(5) Avoid very high doses in hyporesponsive patients.

Brendan Barrett

Dr. Brendan Barrett is a graduate of University College Cork, Ireland. He is currently a nephrologist with Eastern Health in St. John's, Newfoundland. He is a Professor of Medicine at Memorial University of Newfoundland and Provincial Medical Director of the Kidney Program. Apart from clinical work, Dr. Barrett is involved in the undergraduate and postgraduate curricula at Memorial University's Medical School. He also teaches in a graduate degree program for Clinical Epidemiology.

His current research interests include Contrast-Induced nephropathy, Care of patients with chronic kidney disease, Experiences of Patient Life on Hemodialysis, Clinical Trials in Hypertension and a range of health care delivery projects with emphasis on Evaluation of Health Care Programs.

Wednesday PM - Track 2

Targeting the Brain

The Brain: The Next Frontier

Laurent Lecanu[1,2], [1]The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; [2]McGill University Health Centre, Department of Medicine, Quebec, Canada.

Despite billions of dollars spent in drug development, developing drugs aiming at the central nervous system suffers from one of the highest failure rate. The main reason lies in the blood-brain barrier which represents a hurdle that has proven, so far, very hard to jump. The blood-brain barrier is a very complex structure that combines a particular cellular architectonic, a high metabolism rate and a specific pharmacology relying on various transporter systems that makes it a very efficient exclusion system for most of the drugs. However, very little is known about the physiology of the blood-brain barrier and such lack of knowledge certainly hampers the efforts made to overcome this obstacle. Various approaches are currently being used, from using the transporters located in the blood-brain barrier to nanotechnologies and although they could be regarded as very attractive, the current technologies may reach very quickly the limits of their application range. Overcoming the blood-brain barrier is one of the most exciting but difficult challenge faced nowadays by drug developers, and the future of the whole CNS R&D depends on how well we respond to it.

Vectors Targeting Transferrin Receptors: Do they Cross the Blood-Brain Barrier?

Frédéric Calon, Faculty of Pharmacy, Université Laval, Québec, Canada.

The majority of therapeutic molecules do not enter the brain due to the blood-brain barrier (BBB), which has become a limiting step in CNS drug development. The development of vectors traversing the BBB remains a major pharmaceutical challenge. Several lines of evidence suggest that monoclonal antibodies (MAbs) targeting receptor-mediated transcytosis system located on brain endothelial cells can be transported across the BBB. However, few direct evidence of vector transcytosis into the brain have been published.

We have characterized the brain distribution of two MAbs targeting the mouse transferrin receptor (TfR) (clones Ri7 and 8D3) compared to control IgGs after intravenous injection into mice. Ri7 and 8D3 MAbs were fluorolabeled with either Alexa Fluor (AF) dyes 647 or 750. Intravenous injection of Ri7 or 8D3 MAb coupled with AF750 led to higher fluorescence emission in brain homogenates compared to control IgGs, confirming brain uptake. However, fluorescence microscopy analysis revealed that AF647-Ri7 signal was confined to brain cerebrovasculature, colocalizing with an antibody against collagen IV, a marker of basal lamina. Confocal microscopy analysis confirmed the delivery of injected Ri7 MAb into brain endothelial cells using the pericyte marker anti-α-smooth muscle actin (α-SMA), the endothelial marker CD31 and the collagen IV antibody. No evidence of colocalization was detected with neurons or astrocytes identified using antibodies specific for neuronal nuclei (NeuN), or glial fibrillary acidic protein (GFAP), respectively.

In summary, our data show that anti-TfR vectors injected intravenously readily accumulate into brain capillary endothelial cells but not in neurons or astrocytes. Although Ri7 and 8D3 MAbs may not fully cross the BBB in high number, they nevertheless display strong drug targeting potential for endothelial TfR-expressing cells.

Frédéric Calon

Dr Calon's (BSc Biochemistry, BPharm Pharmacy, and PhD Pharmacy at Université Laval) Ph.D. studies on levodopa-induced dyskinesias in Parkinson disease were supervised by Drs Thérèse

Di Paolo and Paul J Bédard and were recognized by the Gold Medal from the Governor General of Canada in 2001. Being both a biochemist and a pharmacist, Dr Calon realized early how the blood-brain barrier (BBB) was a huge health care problem. Supported by a prestigious Senior Research Fellowship from the Canadian Institutes of Health Research, he first went to the laboratory of Dr. William Pardridge at UCLA to learn the latest brain drug delivery techniques. Then, Dr. Calon extended his expertise on Alzheimer's disease by joining the research team of Dr. Greg Cole at UCLA, where they made high-impact discoveries on the role of omega-3 fatty acids in neurodegenerative diseases.

Dr Calon was recruited at Université Laval in July 2003 and he was promoted to Associate Professor at the Faculty of Pharmacy in 2007. He now leads research project on Alzheimer's disease, Parkinson's disease, essential tremor and on the delivery of drug through the blood-brain barrier. His laboratory is located at the Centre Hospitalier Universitaire de Québec (CHUQ) within its research facility located at Centre Hospitalier de l'Université Laval (CHUL). He has published over 58 papers, including in highly-cited journals in the field of neuroscience. His research team currently comprises 8 graduate students, 2 postdoctoral fellows and 2 research professionals.

Development of New Platform Technology Incorporating Peptides into Small and Large Therapeutics for the Treatment of Brain Disorders

Reinhard Gabathuler, Cydweli Consultants Inc., Montreal, QC, and biOasis Technologies Inc., Vancouver, BC, Canada

The blood–brain barrier (BBB) is formed by the special nature of the endothelial cells of the brain capillaries characterized by tight junctions between cells and a high expression of efflux pumps only allowing the brain access to nutrients necessary for cell survival and function. These properties of the BBB result in the incapacity of small and large therapeutic compounds to reach the brain at therapeutic concentrations. Various strategies are now being developed to enhance the amount and concentration of these compounds in the brain parenchyma.

The development of new technologies such as peptide vectors has the potential to achieve the delivery of active agents in therapeutic concentrations across the BBB to treat brain diseases such as brain primary and metastatic cancers and neurodegenerative disorders.

In this presentation, the design of new active peptides and development of new peptide and protein vectors for drug brain delivery using physiological approaches will be addressed. The development of receptor ligand such a protein melanotransferrin (p97) by biOasis Technologies Inc. and a peptide vector Angiopep by Angiochem Inc. as vector for brain delivery will be presented.

Small chemotherapeutic (doxorubicin) and lysosomal enzymes are able to cross the BBB after conjugation to p97 and reach therapeutical concentration in the brain parenchyma in animal models. Angiopep was developed by Angiochem Inc. and incorporated in a small anticancer agent (paclitaxel) (ANG1005 now GRN1005) has shown efficacy in Phase I/II clinical trials for the treatment of brain cancers.

In conclusion, the new chemical entity ANG1005 now GRN1005 conjugate between Angiopep and Paclitaxel is the first of such designed agents to be validated for the treatment of human brain cancers and opens the door for such approaches.

Reinhard Gabathuler

Dr Gabathuler obtained his PhD at the Université de Lausanne, Switzerland, in 1982, and completed postdoctoral studies at the University of Washington, Seattle. Over the years, he has held various research positions at the Swiss Institute for Experimental Cancer Research, Lausanne; the Ludwig Institute for Cancer Research at the Karolinska Institutet, Stockholm, Sweden; and the Biotechnology Laboratory of the University of British Columbia, Vancouver, Canada. His research interests have led him to study the role of phospholipids in the function of ATPases and electrogenic pump to the regulation of intracellular transport of specific receptors and proteins involved in immune-recognition and in development of tumors. His research and development (R&D) of a new vector for delivery of therapeutics to the brain led to the creation of Synapse Technologies Inc., where he became its Vice President of Research. The company was later acquired by BioMarin Pharmaceutical Inc., where Dr Gabathuler assumed the position of Vice President of Brain Research.

Dr Gabathuler joined AngioChem Inc. in 2004 as its Chief Scientific Officer and has applied his

extensive knowledge in biochemistry, cell biology, and immunology to directing the R&D programs, advancing the company's products to IND application and clinic.

As the VP Research and Development of biOasis Technologies Inc., Dr Gabathuler is now involved in the development of new peptide vectors for the brain delivery of biologics based on a protein melanotransferrin (p97). These properties were discovered and characterized at UBC by the group of Wilfred Jefferies and now developed in biOasis Technologies Inc.

Drug Transport in Glial Cells: Contribution to the Neurovascular Unit Barrier Function

Reina Bendayan, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada

Limited drug penetration is an obstacle that is often encountered in the treatment of CNS diseases including human immunodeficiency virus type-1 (HIV-1) encephalitis (HIVE). One mechanism that may contribute to this phenomenon is the expression of ATP-binding cassette (ABC) membrane-associated drug efflux transporters [i.e., P-glycoprotein (P-gp), Multidrug Resistance-Associated Proteins (MRP/Mrp), Breast Cancer Resistance Protein (BCRP; also known as ABCG2)] at the blood-brain barrier (BBB). We have demonstrated that ABC transporters are not only expressed in brain microvessel endothelial cells which primarily constitute the BBB but also in glial cells, the primary target of HIV infection in the brain. We propose that these cells may also contribute to the low accumulation and altered distribution of several therapeutic compounds including antiretroviral drugs in the brain by serving as a "secondary barrier" to drug permeability. In addition, pathological factors such as inflammation (i.e., cytokine release) and oxidative stress associated with HIV-1 infection may alter the expression of ABC transporters and lead to changes in CNS antiviral drug uptake and/or distribution. This presentation will discuss the complexity of drug-transporter interactions in glial cells in the context of HIV-associated immune and oxidative stress responses.

Reina Bendayan

Reina Bendayan is a Professor and Associate Dean Graduate Education, Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto. After obtaining a Bachelors of Sciences in Pharmacy and a Hospital Pharmacy Residency Program at the University of Montreal, Reina Bendayan completed a Doctor of Pharmacy at the University of Florida and a three year Medical Research Council Post-Doctoral Fellowship Program in Clinical Pharmacology and Membrane Cell Biology at the University of Toronto. Dr. Bendayan's research program at the University of Toronto is primarily focused on Membrane Transport and Therapeutics with an emphasis in the field of HIV/AIDS Antiviral Drug Transport. Her research is primarily funded by the Canadian Institutes of Health Research, Canadian Foundation for AIDS Research and the Ontario HIV Treatment Network, Ministry of Health of Ontario. She has participated to the organization of several Workshops and Symposia for International and National Pharmaceutical Sciences Conferences as well as Gordon Conferences on "Barriers of the CNS". Dr. Bendayan was recently elected FELLOW of the American Association of Pharmaceutical Sciences and has received a five-year Career Scientist Award from the Ontario HIV Treatment Network.

Wednesday PM - Track 3

NHPs and Cancer

Selective Induction of Autophagy and Apoptosis through Treatment with Dandelion Root Extract in Human Pancreatic Cancer (NHPRS-01-01)

Madona Chochkeh, Pardis Akbari Asl, Pamela Ovadje, Siyaram Pandey.
University of Windsor, Windsor, ON, Canada.
e-mail: spandey@uwindsor.ca

Pancreatic cancer, one of the most deadly cancers with 100% mortality rate, has no early signs or symptoms and it is estimated that 3900 Canadians will die of this disease in 2010. Current pancreatic cancer treatments include surgery, radiation therapy, hormonal therapy, chemotherapy or some combination of these treatments. Natural compounds have also been used to treat many ailments in the past, including cancer. However, all current treatments have lacked selectivity in targeting cancer cells. *Taraxacum officinale*, also known as dandelion, is one of the many natural compounds used in herbal medicine. Traditionally, it has been used for inflammation modulation, digestive stimulation and liver functioning. We have previously shown that dandelion root extract (DRE) selectively induces apoptosis in human leukemia cells (Ovadje et al, 2011). In this study, we investigated the effect of DRE on human pancreatic cancer cells. Our results show that the human pancreatic cell lines, BXPC3 and PANC-1, are sensitive to aqueous dandelion root extract and this extract induces selective apoptosis as indicated by nuclear condensation and externalization of the phosphatidylserine in a dose and time dependent manner. We also observed that DRE causes pro-death autophagy and collapse of the mitochondrial membrane potential in these cells. Normal Human Fibroblasts were resistant at similar doses. Overall, we demonstrate that DRE has the potential to induce apoptosis in human pancreatic cancer cells with no effect on non-cancerous cells. This will provide a basis upon which further research in the field of cancer treatment through DRE can be executed.

Reduction of Colorectal Hyperplasia by Immunomodulatory Effects of *Cistanche deserticola* Extract in a Mouse Model of Colon Cancer (NHPRS-01-02)

Yamin Jia, Qiunong Guan, Yuhai Guo, and Caigan Du.
[1]Department of Urologic Sciences, University of British Columbia, Vancouver, Canada; [2]College of Agronomy and Biotechnology, China Agricultural University, Beijing, China.
email: caigan@interchange.ubc.ca

Colorectal cancer is a leading cause of cancer-related death in the developed world, and may be progressed from hyperplastic polyps. *Cistanche deserticola* (*C. deserticola*) is one of common medicinal herbs in traditional Chinese medicine for many health problems including irritable bowel syndrome or constipation. This study was designed to test the efficacy of water-extract of *C. deserticola* in the prevention of colorectal cancer in a mouse model. Water-extract of *C. deserticola* was prepared by boiling its stem powder in distilled water. *Tgfb1Rag2* null mice were used as a mouse model of colorectal cancer. Cancerous lesions in the cecum and colon were examined by histology. Here we showed that orally feeding of water-extract of *C. deserticola* significantly reduced the number of mucosal hyperplasia and intestinal helicobacter infection in mice. The beneficial effect of herb extract treatment correlated with significant stimulation of the immune system, evidenced by the enlargement of the spleens with increased number of splenic macrophage and natural killer cells, and with more potent cytotoxicity of splenocytes. *In vitro* addition of water-extract of *C. deserticola* enhanced the cytotoxicity of naïve splenocytes against a human colon cancer cell line, and stimulated the expression of nitric oxide/nitric oxide synthase II and phagocytosis in macrophage cultures. In

conclusion, our data indicate that oral administration of *C. deserticola* extract reduces cancerous hyperplastic polyps and helicobacter infection in mice by its immune-stimulatory activity, suggesting that this medicinal herb has potential in the prevention of colorectal cancer and perhaps other intestinal inflammation disorders as well.

A Novel Synthetic C1 Analogue of 7-deoxypancratistatin Selectively Induces Cytotoxicity in Cancer Cells, Exhibits Enhanced Activity with Curcumin and Tamoxifen, and Reduces Human Tumour Growth in Xenograft Models (NHPRS-01-03)

Dennis Ma[1], Phillip Tremblay[1], Kevinjeet Mahngar[1], Pardis Akbari-Asl[1], Carly Griffin[1], Jonathan Collins[2], Tomas Hudlicky[2], and Siyaram Pandey[1] .
[1]Department of Chemistry and Biochemistry, University of Windsor, Windsor, Ontario, Canada, [2]Chemistry Department and Centre for Biotechnology, Brock University, St. Catharines, Ontario, Canada, e-mail: spandey@uwindsor.ca

For centuries, a plethora of natural compounds have been employed for the treatment of numerous ailments. The natural compound pancratistatin (PST), from the *Hymenocallis littoralis* plant, specifically induces apoptosis in a variety of cancers. Unlike most chemotherapies, PST is non-genotoxic and markedly less toxic to non-cancerous cells. However, preclinical and clinical work is limited by low availability in its natural source and difficulties in its chemical synthesis. Previously, we have found that JC-TH-acetate-4 (JCTH-4), a synthetic C1 acetoxymethyl derivative of 7-deoxypancratistatin, to have anti-cancer activity comparable to native PST in human leukemia cells. In this study, we evaluated the efficacy of JCTH-4 on aggressive cancer cells, including osteosarcoma, pancreatic adenocarcinoma, glioblastoma, breast adenocarcinoma, and colorectal cancer cells. All these cancer cells were susceptible to JCTH-4-induced apoptosis, mechanistically acting on cancer cell mitochondria; JCTH-4 decreased mitochondrial membrane potential, and increased production of reactive oxygen species and caused release of apoptogenic factors in isolated mitochondria. Interestingly, cytotoxicity of JCTH-4 was synergistically enhanced by the natural compound curcumin in cancer cells, including notoriously chemoresistant osteosarcoma and colorectal cancer cells. Similarly, tamoxifen enhanced the activity of JCTH-4. Furthermore, JCTH-4 was able to reduce growth of human p53 positive and negative colon tumours in nude mice. JCTH-4 exhibited minimal toxicity in various non-cancerous cell lines and in nude mice. Hence, JCTH-4 is a novel compound capable of selectively targeting cancer cells, alone and in combination with curcumin and tamoxifen, and may serve as a more safe and effective alternative to current cancer therapies.

Evaluating the Efficacy of Dandelion Root Extract in Human Chronic Myelomonocytic Leukemia Cells (NHPRS-01-04)

Pamela Ovadje[1], Caroline Hamm[2], Siyaram Pandey[1].
[1]Department of Chemistry and Biochemistry, Canada; [2]Windsor Regional Cancer Centre University of Windsor, Windsor, Ontario, Canada, e-mail: spandey@uwindsor.ca

Chronic MyeloMonocytic Leukemia (CMML), one of the most resistant, non-responsive forms of leukemia, is very hard to classify and diagnose as it embodies properties of both myeloproliferative disorders (MPD) and myelodysplastic syndromes (MDS). For this reason, WHO has classified CMML as a "mixed myelodysplastic/myeloproliferative disease". Due to its high resistance to treatment, it is therefore necessary to find a more efficient mode of treating this disease. Natural compounds have been used as anti-oxidant, anti-inflammatory and anti-viral agents in traditional medicine for centuries to treat various ailments. Substantial research has been done to delineate the medicinal components of natural extracts for clinical use, especially for the field of cancer. Our group is studying the anti-cancer properties of dandelion root extract (DRE), which is currently marketed for management of gastrointestinal and liver disorders. We previously reported that aqueous DRE effectively and selectively induces apoptosis in human leukemia (Jurkat) cells in a dose and time dependent manner by rapidly activating the extrinsic pathway of apoptosis. Results show that CMML also responded to DRE in a dose and time dependent manner and apoptosis was measured using standard apoptotic markers. The growth of these cells was halted after DRE had been removed from the media and the loss of mitochondrial membrane potential was observed. A solvent enriched fraction of DRE was able to induce apoptosis in these cells at lower concentrations suggesting that DRE contains components that induce apoptosis selectively in

human leukemia cells presenting a novel non-toxic alternative to conventional leukemia and CMML therapy.

Targeting Mitochondria in Cancer Cells to Induce Apoptosis by Pancratistatin, a Natural Products from Spider Lily (NHPRS-01-06)

Siyaram Pandey[1], Carly Griffin[1], Sudipa Chatterjee[1], Dennis Ma[1] and James McNulty[2]. 1University of Windsor, 2McMaster University, University of Windsor, Windsor, Ontario, Canada.
e-mail: spandey@uwindsor.ca

Mitochondria play a central role in apoptosis and is therefore the focus of recent research efforts as selective target for killing cancer cells Mitochondrial and metabolic differences in cancerous and non-cancerous cells my provide selective targets to destabilize cancer cell mitochondria with no toxic effects on non-cancerous cells. Exploration of natural compounds that could selectively target cancer cells may provide an alternative to genotoxic chemotherapies. We have shown that Pancratistatin (PST), a natural compound isolated from the Hawaiian spider lily by Petit in 1992, induces apoptosis in numerous human cancer cell lines, including colon, breast, leukemia and melanoma, with no toxicity to non-cancerous cells. We have demonstrated the non-genotoxic nature of PST. Our results suggest that PST's selective killing of cancer cells may arise due to its interaction with mitochondria related apoptotic factors that are present exclusively (overexpressed or exposed) in cancer cells. Our experiments showed that mtDNA-depleted □0 cells were resistant to PST, indicating direct involvement of mitochondrial proteins in PST-induced apoptosis. Furthermore, we observed that PST treatment causes a) reduces ATP biosynthesis, b) selectively increases mitochondrial membrane permeability and c) causes generation of reactive oxygen species from mitochondria, when applied to human tumour cell lines or their isolated mitochondria but not in normal cells. We have also demonstrated that PST is well tolerated in mice, and effectively reduces human tumour xenotransplants in nude mice. This work opens up a new window of opportunity for developing non toxic anti cancer therapies.

Wednesday PM - Track 4

Metabolism and Diabetes

AD03: An Alternative Treatment to Improving Hyperglycemia and Hyperinsulinemia in a Diet-induced Obese Model (NHPRS-02-01)

Despina Harbilas[1,2,3,4], Antoine Brault[1,2,3,4], Diane Vallerand[1,2,3,4], Ammar Saleem[2,5], John T. Arnason[2,3,5], Lina Musallam[1,2,3,4], Pierre S. Haddad[1,2,3,4].
[1]Laboratoire des produits de santé naturels et maladies métaboliques, Département de pharmacologie, Faculté de médecine, Université de Montréal, [2]Équipe des IRSC sur les médecines autochtones antidiabétiques, [3]MDRC, [4]INAF, [5]Department of Biology, University of Ottawa
Email: dharbilas@yahoo.ca

Our team (CIHR-TAAM) conducted ethanobotanical studies in the Cree of Eeyou Istchee communities of Northern Quebec and identified 17 plants with anti-diabetic potential. Previous *in vitro* screening studies revealed that one of these plants, AD03, produced strong anti-diabetic effects. Therefore, AD03 was further investigated in a mouse model of diet-induced obesity and T2D. In the prevention study C57/BL6 mice were subjected to high fat (HF) diet for eight weeks to which AD03 was incorporated at 125 and 250 mg/kg. In the treatment study, the mice were subjected to HF diet for sixteen weeks. AD03 was introduced in the HF diet for the last eight weeks and tested at 125 and 250 mg/Kg. Prevention study: AD03 at 250 mg/Kg gradually but significantly decreased whole body and retroperitoneal fat pad weights and improved circulating adipokine levels as compared to HF cognates. No significant effects were observed on glycemia and insulinemia. Treatment study: AD03 significantly and dose-dependently improved glycemia and insulinemia levels, circulating adipokine levels as well as the G/I index (indicator of insulin resistance) when compared to HF controls. However, AD03 improvement effect on body weight or retroperitoneal fat pad weight was not as pronounced as in the prevention study. In both the prevention and treatment study, no statistical difference was observed in water or food intake. AD03 thus exhibits promising anti-diabetic and slight anti-obesity effects. Mechanisms remain to be elucidated in the liver, muscle and adipose tissue, all targeted by T2D and obesity. Funded by the CIHR.

Local Administration of Plants in Treating Diabetes Mellitus: An Ethnopharmacological Study from the Narayanganj District of Bangladesh (NHPRS-02-02)

MD. Ariful Haque Mollik[1], MD. Rohmul Faruque[1], Khadiza Akter[1], MD. Badruddaza[2], Romeo McField[3], Dilara Ferdausi[4].
[1]Department of Epidemiology, Biostatistics, Community Nutrition and Noncommunicable Diseases, Peoples Integrated Alliance, Dhaka, Bangladesh [2]Department of Biotechnology & Genetic Engineering, North South University, Dhaka, Bangladesh [3]Department of Epidemiology, Biostatistics, Community Nutrition and Noncommunicable Diseases, Practical Academy on Wise Education and Research Foundation, Dhaka, Bangladesh [4]Department of Molecular Biology, University of Skövde, Skövde, Sweden

Uses of plants in the indigenous cultures of developing countries are numerous and diverse. This indigenous knowledge evolved for along time through trial and error. Though the majority of people in the Narayanganj district of Bangladesh rely on plants to manage of diabetes mellitus, a lot the indigenous knowledge largely remains undocumented. An ethnopharmacological study was conducted on plants used to manage of diabetes mellitus at the Narayanganj district. Herbalists administered treatment mainly by concoctions, decoctions majorly through oral and dermal routes to treat of diabetes mellitus. It was also noted that most herbalists preferred using herbal preparations consisting of more than one plant to treat of diabetes

mellitus. During interviews with herbalists from the Narayanganj district, eleven plants were reported to be useful in management of diabetes mellitus. All plant samples were later identified at the Bangladesh National Herbarium. The plant names used to treat diabetes mellitus included *Coccinia grandis* (L.) Voigt, *Nigella sativa* L., *Agaricus campestris* L., *Olea europaea* L., *Grewia asiatica* L., *Punica granatum* L., *Murraya koenigii* (L.) Spreng., *Abroma augusta* (L.) L.f., *Glycosmis pentaphylla* (Retz.) DC., *Ficus racemosa* L., and *Lepidagathis hyalina* Nees. Preliminary phytochemical and bioactivity analyses of these plants showed that they were active against of diabetes mellitus. Information on indigenous use of plants has led to discovery of many medicines in use today. It is important to conduct more studies towards elucidation of the pharmacological constituent(s) responsible for the hypoglycemic activity and evaluate their potential in the treatment of diabetes mellitus.

Main Considerations about Interactions between Herbal Remedies and Medicinal Drugs, Reports in Cuba (NHPRS-02-03)

Diadelis Remirez[1] Giselle Gimenz[2].
[1]National Center for State Quality Control of Drug.
[2] National Centre for Pharmacoepidemiology, Playa, Habana, Cuba.

The use of herbal medicine to treat a wide range of conditions is rapidly leading to increades intake of phytochemicals. This is one of the main reasons for reinforcing the surveillance of the safety, efficacy and quality control of traditional and complementary medicines. Herbal preparations can interact with a drug at different levels. Cuba has a Center in charge of pharmacovigilance for the detection, assessment understanding and prevention of adverse effects or any other possible drug related problems. It includes herbal, traditional and complementary medicines, blood products, biological and medical devices etc. This work will describe the updated interactions between herbal and conventional medicines and reports about the side effects of phytomedicines and drugs in Cuba as well as the proposed mechanism will be also included. If potential drug interactions are to be predicted, it is essential that the ability of herbal products to interfere with drug-metabolizing enzyme systems is fully established. The importance to evaluate potential drug interactions prior to market approval as well as during the post marketing period are also analyzed in this work.

Ethnobotany and Pharmacology of Anti-Inflammatory Medicines Used by the Q'eqchi' Maya of Belize (NHPRS-02-04)

Brendan Walshe-Roussel[1], Christine Choueiri[2], Max London[1], Ammar Saleem[1], Federico Caal[3], Victor Cal[3], Todd Pesek[4], Tony Durst[2], John Thor Arnason[1].
[1]Department of Biology, University of Ottawa, Ottawa, Ontario, Canada.[2]Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada.[3]Belize Indigenous Training Institute (BITI), Punta Gorda, Toledo District, Belize. [4]Department of Health Sciences, Center for Healing Across Cultures, Cleveland State University, Cleveland, Ohio, USA .

Inflammation has been implicated as a causative or contributing factor in the pathophysiology of many disorders and a wide range of symptoms are associated with inflammatory conditions. As such, immunomodulation represents an important target in attempts to understand and treat many categories of illness. Indigenous pharmacopeias recognize the important role of inflammation in disease, and the Q'eqchi' Maya healers of Belize possess a practical understanding of a large number of immunomodulatory botanicals. Ethnobotanical interviews were held with 5 members of the Q'eqchi' Maya Healers Association using a list of 14 inflammatory symptom categories and one hundred and seven plant species were collected from primary and secondary semi-evergreen rainforest in the Maya mountains of S. Belize. Ethanolic extracts of fifty-five species were assayed for anti-inflammatory activity in a LPS-stimulated THP-1 monocyte assay. Among species assayed, 76% demonstrated significant anti-inflammatory activity relative to the vehicle control, and three species displayed anti-inflammatory activity equal to that of the parthenolide positive control. In addition, several sesquiterpene lactones isolated from the traditionally used *Neurolaena lobata* exhibit potent anti-inflammatory activity. These results demonstrate that plants used by the Q'eqchi' Maya Healers Association for the treatment of inflammatory-related symptoms do indeed possess immunomodulatory properties, and elucidating the active principles of these species can yield compounds with novel bioactivities.

Beneficial Effects in the Liver of Antidiabetic Plants used in Traditional Medicine by the Cree of Bay James in Canada (NHPRS-02-05)

Abir Nachar[(1,3)], Ammar Saleem[(2)], Lina Musallam[(1,3)], Diane Vallerand[(1,3)], Louis Lavoie [(1,3)], John T. Arnason[(2)], Pierre S Haddad[(1,3)].
[1]Natural Health Products and Metabolic Diseases Laboratory, Dept. of Pharmacology, Université de Montréal, Montréal, Québec, Canada; [2] Phytochemistry, Medicinal Plant and Ethnopharmacology Laboratory, Dept. of Biology, University of Ottawa, Ottawa, Ontario, Canada; [3] Canadian Institutes of Health Research Team in Aboriginal Antidiabetic Medicines and Montreal Diabetes Research Center

The liver plays an essential role in the regulation of glucose homeostasis and in the pathogenesis of type 2 diabetes mellitus. This study sought to determine the potential beneficial effect of putative antidiabetic medicinal plants used by the Eastern James Bay Cree of Northern Quebec (Canada) on the activity of key enzymes of gluconeogenesis and glycogen synthesis. Glucose-6-phosphatase (G6Pase) activity was measured by glucose oxidase enzymatic assay and glycogen synthase (GS) activity was assessed by the amount of radioactive UDP-glucose incorporated into glycogen in hepatic cell lines. The phosphorylation of AMP-dependent protein kinase (AMPK) and Glycogen synthase kinase-3 (GSK-3), the two key kinases which control the two pathways for glucose homeostasis, were probed by Western blot. Eight of the tested seventeen plant extracts significantly inhibited G6Pase, the key enzyme for gluconeogenesis, and activated glycogen synthase, the enzyme responsible for storage of glucose as glycogen. Phosphorylation of AMPK and GSK-3 were increased significantly by several active extracts. The most active extract showed 48 % of inhibition for G-6Pase and over 10-fold activation of GS. Bioassay guided fractionation of this extract identified the hexane fraction as the most active (50 % inhibition of G6Pase; very high activation of GS). The subfractionation of the hexane fraction yielded eight pure compounds, three of which are active. Several Cree antidiabetic medicinal plants modulate G6Pase and GS. The crude extract and the pure compounds of the most active species have potential to treat type 2 Diabetes.

Evaluate and Compare the Anti-Diabetic Potential of Ethanol and Water Extracts of 17 Plants used by the Eeyou Istchi Cree First Nations of Northern Quebec (NHPRS-02-06)

Nan Shang [1,2], Lina Musallam[1,2,] Diane Vallerand [1,2],Brendan Walshe-Roussel [2,3], John T. Arnason[2,3] , Pierre S. Haddad[1,2].
[1]Natural health products and metabolic disease laboratory, Département de pharmacologie, Faculté de médecine, Université de Montréal; [2]Canadian Insititutes of Health Research Team in Aboriginal Antidiabetic Medicines and Montreal Diabetes Research Center; [3]Medicinal Plant and Ethno-pharmacology laboratory, Department of Biology, University of Ottawa.

The age-adjusted prevalence of diabetes in the Cree of Eeyou Istchee (northern Quebec) reached 29% in 2009. Our group (CIHR-TAAM) identified 17 plants used by the Cree to treat symptoms of diabetes and screened their 80% hydrated ethanol extracts (EE), using an in vitro bioassay platform. Although, common phytochemical approaches to extract active principles of promising plant species usually uses EE, traditional preparations are often based on hot water extractions (HWE). We thus compared these two extraction methods on the anti-diabetic potential of the 17 Cree plants. Two main bioassays routinely applied in our laboratory were used: 1) potentiation of adipogenesis by measuring accumulation of triglycerides (in 3T3-L1 cells), and 2) inhibition of hepatic glucose production by measuring inhibition of glucose-6-phosphatase activity (G-6Pase; in H4IIE cells). Our results show that 10 out of the 17 HWE plants lost either the potentiating or the inhibitory effects on adipogenesis in 3T3-L1 cells induced by their EE counterparts. For glucose production, only 7 out of 17 HWE have lower G6Pase activity compared to EE counterparts. The data confirm that the method of extraction is a significant determinant of the biological activity of a medicinal plant. Although EE better extracts phenolics, traditional medicinal plant preparations of aboriginal healers might differ in the HWE conditions and this could yield more concentrated solutions. Changes in the quality and quantity of extract components as well as underlying mechanisms of action remain to be elucidated.
Funded by CIHR and the China Scholarship Council.

Wednesday PM - Track 3

Cognition and Neurodegenerative Disease

Osha (*Ligusticum* spp): Potential in an Underrepresented North American Medicinal Plant (NHPRS-03-01)

Christina E. Turi[1] and Susan J. Murch[2].
Department of [1]Biology and [2]Chemistry, University of British Columbia, Okanagan Campus, Kelowna, British Columbia, Canada,.
e-mail: susan.murch@ubc.ca

Ligusticum species belong to the family Apiacieae (carrot family). In North America, there are 12 species of *Ligusticum*. Among these, *Ligusticum porteri* and *Ligusticum canbyi* have purported ceremonial uses to treat psychological illnesses of unknown or spiritual origin. Opler (1923) reports that the Tonkwa require that one must possess *L. porteri* in order to partake in the peyote ceremony, while Turner et al. (1980) found that the Okanagan-Colville peoples would use smoke produced from *L. canbyi* to treat individuals believed to be in a trance, possessed by spirits, or who are ceremonially unconscious. Although species of *Ligusticum* found within Traditional Chinese medicine are well studied with known pharmacologically active compounds, far less information upon North American species exists. Consequently, specific phytochemicals responsible for *L. porteri* and *L. canbyi's* traditional use as a psychoactive have not been fully identified or characterized. Our recent research has been focused on understanding the neurologically active phytochemistry of *Ligusticum* species. Although further investigation is still needed, North American species of *Ligusticum* posses a wealth of research potential, which in turn may lead to the discovery of novel compounds, mechanisms of biological activity and/or new medicinal uses.

In vitro Assessment of Neuroactivity of Canadian Conifers and Phtyochemicals: A Potential Source of Natural Products for the Canadian Forest Products Industry (NHPRS-03-02)

Andrew Waye[1], Jose-Antonio Guerrero-Analco[1], Ammar Saleem[1], Malar Annal[1], Andrew Tang[1], Mark Hewitt[2], Craig Milestone[2], Deborah MacLatchy[3], Vance L Trudeau[1], John T. Arnason[1].
[1]Department of Biology, University of Ottawa, Ottawa, ON; [2]Canada Centre for Inland Waters, Environment Canada, Burlington, ON;. [3]Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada.

Canadian plants contain substances that interact with the neurophysiology of vertebrates. The ability of phytochemicals to mediate physiological processes provides researchers the possibility to discover novel drugs or natural products to treat a variety of neurological diseases. We hypothesize that chemicals in Canadian conifers may interact with key neurotransmitter systems in the brain that are indicated in such diseases as anxiety, epilepsy, or depression. We report that organic solvent extracts of nine Eastern Canadian conifers (balsam fir, black spruce, white spruce, white pine, Eastern hemlock, common juniper, white cedar, jack pine, and tamarack larch) have the potential to interact with the gamma-aminobutyric acid (GABA) and dopamine (DA) systems. Drugs that interact with the GABAergic system are used as anticonvulsants or anxiolytics. Their mode of action is often by potentiating GABA activity by binding to the GABA-A benzodiazepine receptor (GABA(A)-BZD) in the brain. Interactions with glutamic acid decarboxylase (GAD) and GABA transaminase (GABA-T), the enzymes that synthesize and metabolize GABA respectfully, are also drug targets. Drugs that interact with the DAergic system are often used to treat depression or addiction. These drugs often inhibit monoamine oxidase (MAO), an enzyme responsible for metabolizing DA. We screened conifer extracts and pure compounds found in Canadian conifers in vitro using the GABA(A)-BZD receptor binding assay as well as GAD, GABA-T, and MAO enzyme assays. These results show that Canadian conifers contain substances capable of interacting with the DAergic and GABAergic systems in vertebrates, highlighting their potential

for future drug or natural product discovery.

Neuroprotective Effects of Water Soluble CoQ10 in a Paraquat Induced Rat Model of Parkinson's Disease: The Role of Neurotrophic Factors (NHPRS-03-03)

S. Pandey[1], K. Facecchi[1a], J.K. Sandhu[2], M. Sikorska[2], M. Somayajulu-Nitu[1], P. Dadwal[1], J. Cohen[1].
[1]University of Windsor, Windsor ON, [2]Ist. Biol. Sci. National Research Council of Canada. Ottawa, ON.

Parkinson's disease (PD) is a neurodegenerative disorder affecting approximately 4 million people worldwide. It results from the progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). Oxidative stress and mitochondrial dysfunction have been implicated in the patahology of PD. Currently there is no treatment to halt the progression of PD. Recently we have shown that water-soluble coenzyme Q10 (WS-CoQ10, NRC technology licensed to Zymes LLC) prevents the loss of dopaminergic neurons in the SNpc and ameliorates the symptoms of paraquat-inducted PD in the rat model. We have observed that there is activation of astroglia in the brains of WS-CoQ10 treated animals. Whether this activation plays a role in protecting SNpc neurons is unknown. It is possible that activated astroglia cells produce neurotrophic factors that may protect neurons under oxidative stress. We used immunohistochemistry and western blots to analyze brain tissue samples from paraquat treated PD rats fed with either the vehicle or WS-CoQ10 for DA neurons, activation of astroglia and levels of various neurotrophic growth factors. We observed significant protection of DA neurons in the SNpc, activated astroglia and increased levels of brain derived neurotrophic factor (BDNF) and glial derived neurotrophic factor (GDNF) in WS-CoQ10 fed rats. These results indicate that WS-CoQ10 treatment could activate astroglia that produce high levels of BDNF and GDNF and could provide protection to the DA neurons against paraquat toxicity. Therefore, this formulation of CoQ10 may be used to slow or even halt the progression of Parkinson's disease.

***Panax ginseng* Augmentation: Effects on Depressive and Negative Symptom Clusters and Neurocognition in Schizophrenia (NHPRS-03-04)**

Veronica Sanchez[1] Simon Chiu[2,3] Zack Cernovsky[2] John Copen[4] Mariwan Husni[5].
[1]Faculty of Science, University of Western Ontario, London, Ontario; [2]Department of Psychiatry, University of Western Ontario, London, Ontario; [3]Lawson Health Research Institute, London, Ontario; [4]Department of Psychiatry, University of British Columbia, Vancouver, BC , Canada; [5]Department of Psychiatry, Imperial College of London, Faculty of Medicine, United Kingdom.

Introduction: Converging evidence suggests neuroactive steroids are involved in schizophrenia. No controlled studies have been conducted with phyto-neurosteroid herbal supplement, Panax Ginseng as augmentation strategy in schizophrenia. Ginseng interacts with GABAa, NMDA (N-methyl-D-aspartic) glutamate, dopamine and neuronal nicotinic receptor signalling related to schizophrenia.
Objective: to evaluate the efficacy and tolerability of Panax Ginseng in augmenting antipsychotic therapy in schizophrenic patients exhibiting persistent negative symptoms & neurocognitive impairment.
Method: Randomized placebo-controlled cross-over design. We recruited patients diagnosed as DSM-IV-R schizophrenia with SANS (Scale for Assessment of Negative Symptoms) score >24 and maintained on atypical antipsychotics throughout the study period. The subjects were randomized to either placebo-treated or Ginsana-115 (Boehringher-Ingelhreim-Pharmaton, Switzerland) 200 mg or 100 mg po od. We administered computerized-Neuro-cognitive Screening (NCS), SANS, Brief Psychiatric Rating scale (BPRS), HAM-D (Hamilton-Depression-Rating-Scale) at 2, 5, 8 wk intervals. Safety was monitored with adverse events checklist. Results: We randomized 65 subjects: 39.6 +/-11.9 yrs. Between-subject t-testing showed Ginsana-115 200 mg significantly ($p< 0.05$) reduced Flat-Affect subscale SANS. PG 200 mg reduced HAM-D & BPRS ($p< 0.05$). The response rate of negative symptoms (reduction > /= 30%) was 50.0% favoring Ginsana-115 200 mg vs. 9.1 % for placebo ($p< 0.03$) and for depressive symptoms, 70.0 % for Ginsana-115 vs. 18.2 % for Placebo ($P< 0.01$). Ginsana-115 at 100 mg improved facial memory of NCS. Responses of Ginsana-115 100 mg were similar to placebo. Side effects were uncommon.
Conclusion: Ginseng appears promising in improving negative & depressive symptoms and well tolerated in schizophrenia. Supported by Stanley Medical Research Institute Grant USA.

Wednesday PM - Track 4

National and International Perspectives

Natural Health Products: Post-Market Perspectives (NHPRS-04-01)

Mano Murty[1].
[1]Marketed Health Products Directorate, Health Canada, Ottawa, ON (A/L 0701A).
Email: mano.murty@hc-sc.gc.ca

Natural health products (NHPs), also known as alternative or complementary medicines, and their widespread usage by the population at large, present a challenge to both health care practitioners and the regulatory agencies. Most consumers of NHPs use them for a variety of ailments and are often unaware that NHPs, like all forms of medications, can present a potential risk to human health, especially in the context of NHPs usage in an unsupervised, self-administered setting. Although the risk of potential health care consequences is believed to be low, certain NHPs have been associated with a number of serious adverse reactions. The challenge for the global regulatory agencies is not only to assess the quality, safety and efficacy of these products at the pre-market submission stage, but to continuously evaluate and re-evaluate the NHPs throughout the products= life cycle. Most countries in the past have regulated NHPs as foods, provided that no medicinal claim was stated on the label. However, some countries, such as Australia and the European Agency have already created an evaluation system to address these products separately. In parallel, Canada has created Natural Health Products Directorate (NHPD) in the Health Products and Food Branch (HPFB). In addition, Marketed Health Products Directorate (MHPD) has created a special post-market section, to address specific post-market surveillance issues concerning NHPs. This presentation will highlight some key post-market activities, with a special focus on adverse reactions, risk communication and risk mitigating strategies involving NHPs.

Positive Interactions between Traditional Chinese Herb and Western Drug (NHPRS-04-02)

Jin-Qi Zeng.
Product Assessment Division, Natural Health Products Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON,
Email: jin-qi_zeng@hc-sc.gc.ca

The presentation consists of three parts with the purpose to illustrate the positive side of the interactions between Chinese herb and western drug. The first part talks about the interaction between traditional Chinese herb under the theory of traditional Chinese medicine (TCM) and its application in processing and combination. The second part talks about the interaction between Chinese herb and western drug focusing on the positive side. The third part gives examples of the finished products of combined Chinese herb and western drug under the regulation of SFDA in China.

Preclinical Safety Testing of "Novel" Natural Health Products for Human Clinical Trial Applications

Katherine D. Neufield, Elise Cogo, Robin J. Marles[1].
[1]Bureau of Clinical Trials and Health Sciences, Natural Health Products Directorate, Health Canada, Ottawa, Ontario.

The development of novel natural health products (NHPs) is seen as a popular means of potentially enhancing existing natural health products or exploring possible new avenues of health research. A "novel" NHP is one with a limited (if any) history of human use that has not been sufficiently studied scientifically. As with a new drug, these novel NHPs may present risks to research participants and the Canadian public that need to be well characterized

prior to conducting human clinical trials. Therefore, as per Good Clinical Practice, an adequate safety evidence package must be considered before a novel NHP can be tested in a clinical setting. As indicated by the Investigator's Brochure section of the *Natural Health Products Regulations*, the requirements for a Clinical Trial Application safety package of a novel natural health product are similar in scope to those outlined by the Therapeutic Products Directorate (TPD) of Health Canada for new drug development. The standards for preclinical safety testing, both *in vitro* and *in vivo*, are outlined in documents published by both Health Canada and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). An overview of the safety requirements and steps in preclinical testing of novel NHPs and the basic toxicology principles behind them will be presented and suggestions will be provided.

NHPs in the Western Pacific and Australia (NHPRS-04-03)

Susan J. Murch and Michael Smith.
Natural Health Products Research Society of Canada

As with other regions of the world, in countries of the Western Pacific Region consumers are increasingly including natural health products and traditional medicines within their health care options. This requires all stakeholders, notably regulators, industry and the research community to evolve as a sector in developments appropriate resource and regulatory frameworks. This region is quite unique internationally since it includes countries with developed cultures and communities in Traditional Medicines (notably China, Japan and Korea) as well as those such as Australia and Malaysia with established regulatory frameworks for natural health products. This presentation with provide information on work underway in the region and explore regional issues and opportunities that will have an impact on the international stage. Particular attention will be given to potential opportunities for collaboration with the Canadian NHP sector with an emphasis on key players notably Australia with a focus on research and innovation.

Wednesday PM

CSPT Award Lectures

Pfizer Senior Scientist Award Lecture

Fetal Alcohol Spectrum Disorders (Ethanol Teratogenicity): Engagement of Pharmacology in Addressing this Public-Health Challenge

James F. Brien, James, Department of Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada

Maternal consumption of alcohol (ethanol) during pregnancy can produce a wide spectrum of teratogenic effects in the embryo/fetus, which manifest postnatally as birth defects, collectively referred to as fetal alcohol spectrum disorders (FASD). The most severe manifestation of FASD appears to be fetal alcohol syndrome (FAS). Brain injury is the most debilitating outcome of ethanol teratogenicity, which is one of the most common causes of mental deficiency in the western world. Currently, there is no established therapeutic intervention for this brain injury. In Canada, the estimated incidence of FASD is about one per 100 live births, which demonstrates that FASD is a substantive public-health challenge. Since 1983, our basic-biomedical research program has focused on the investigation of cellular mechanisms of the brain injury of FAS/FASD (ethanol neurobehavioural teratogenicity) and the discovery of potential therapeutic interventions. The successes of the persistent engagement of our team of principal investigators, graduate students and assistants in research on ethanol neurobehavioural teratogenicity over 25 years include: **1.** establishment of the guinea pig as a reliable experimental animal model; **2.** cellular mechanisms, including the glutamate-NMDA receptor-nitric oxide synthase signaling pathway in the developing hippocampus as a key target, changes in GABA signaling in the developing brain, activation of the maternal and fetal HPA axis, and oxidative stress/neuroapoptosis in the fetal hippocampus; and **3.** interventions to mitigate some of the neurobehavioural deficits. This lecture will focus on several of the highlights of our research accomplishments.

(Supported continuously by Queen's University and the Canadian Institutes of Health Research).

James F. Brien

James F. Brien is Professor in the Department of Pharmacology and Toxicology of the School of Medicine and Director of Research in the Faculty of Health Sciences at Queen's University. Dr. Brien received his B.Sc. and Ph.D. degrees in Chemistry from the University of Windsor, followed by a Postdoctoral Fellowship in Pharmacology at the University of Toronto. He held the position of Scientist with the Addiction Research Foundation of Ontario, Kingston Centre, from 1973 to 1978. Dr. Brien joined the Department of Pharmacology in the Faculty of Medicine at Queen's University as Assistant Professor in 1978, and became Full Professor of Pharmacology and Toxicology in 1986. Currently, he is a member of the Senate of Queen's University.

Dr. Brien has an active, multi-faceted research program in pharmacology and toxicology that, for over 25 years, has focused primarily on Fetal Alcohol Syndrome (FAS) / Fetal Alcohol Spectrum Disorders (FASD), with particular emphasis on the brain injury of FAS / FASD. His research program has been funded continuously by the Canadian Institutes of Health Research (CIHR) and the former Medical Research Council of Canada. Dr. Brien has been involved in several functions of CIHR, including multidisciplinary and transdisciplinary peer-review committees, and University Delegate at Queen's. Currently, he is a member of the CIHR Governing Council.

Piafsky Young Investigator Award Lecture

Dissecting the Isoform-Specific Roles for PI3 Kinases in Rodent and Human Insulin Secretion

Patrick MacDonald, Canada Research Chair in Islet Biology, AHFMR and CDA Scholar

Impaired insulin secretion from pancreatic islets of Langerhans contributes to the development of diabetes. Phosphatidyinositol-3-OH kinases (PI3Ks) contribute to islet cell survival and proliferation, insulin gene expression, and the acute control of insulin secretion in response to increased blood glucose. On this last point there is much debate. Non-selective pharmacological inhibition of PI3K enhances glucose dependent insulin secretion, an effect that would be beneficial in the treatment of diabetes. However, recent genetic models of impaired PI3K signaling implicate this pathway as a positive regulator of insulin secretion since genetic down-regulation of PI3K impairs secretion. Our recent work has focused on dissecting the distinct roles, and molecular mechanisms, by which the type 1 PI3K isoforms regulate insulin secretion from mouse and human islets. We find that the type 1A isoforms p110alpha and p110beta, and the type 1B isoform p110gamma, are expressed in insulin secreting islets. Each isoform plays a distinct and important role in the acute control of insulin secretion: p110alpha is a NEGATIVE regulator by limiting calcium-dependent insulin exocytosis; p110beta is a POSITIVE regulator with a role as a structural adaptor in insulin granule docking; and the G-protein coupled p110gamma is a POSITIVE regulator of insulin secretion by controlling cortical F-actin reorganization and the recruitment of insulin granules to the plasma membrane. Much work remains to determine whether isoform-specific inhibition of PI3Ks would be a useful approach to the treatment of diabetes, and to dissect the functional role(s) of the numerous non-classical PI3Ks that are also present in insulin-secreting cells.

Patrick E. MacDonald, PhD

Patrick MacDonald received his BSc from the University of Western Ontario in 1998. He then pursued graduate research at the University of Toronto where he completed a successful PhD in 2003, obtaining numerous awards. Notable among these were the George I. Ellis Memorial Award for excellence in Pharmacology research at the National Student Research Forum. Patrick then pursued postdoctoral training at Lund University in Sweden, where he held a CIHR postdoctoral fellowship and obtained his first independent research grant from the Novo Nordisk Foundation of Denmark. The group moved to a new laboratory at the University of Oxford in 2005 where Patrick was awarded the European Association for the Study of Diabetes/AstraZeneca Fellowship in Islet Biology.

Patrick was recruited to the University of Alberta and the Alberta Diabetes Institute (ADI) in the summer of 2006. At this time he obtained research support from the CIHR and prestigious salary awards including Scholarships from the Canadian Diabetes Association (CDA) and Alberta Heritage Foundation for Medical Research (AHFMR), and the Canada Research Chair in Islet Biology. Patrick's lab moved into the newly opened ADI facilities in November of 2007, and has grown to include 11 students, postdocs and senior personnel. Patrick is Director of the ADI Cellular Imaging Core, and human IsletCore program.

Work in the MacDonald lab is focused on understanding the downstream targets of metabolic and receptor-mediated signaling pathways that control insulin and glucagon secretion from the pancreatic islets of Langerhans in health and diabetes. In particular, the lab studies the regulation of ion channels and exocytotic processes by intracellular metabolites, signaling molecules, and post-translational modification. A significant effort is devoted to investigating these in the pancreatic islets isolated from human donors in order to gain insight into human diabetes and future treatments.

Thursday AM

CSPT Trainee Oral Presentations

Effect of Human Equilibrative Nucleoside Transporter 1 (hENT1) Expression on Adenosine Production from Neurons (CSPT-1)

Chu S, Parkinson FE.
Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada.
Corresponding Author: umchu2@cc.umanitoba.ca
Conflict of Interest: None declared

Background: Adenosine is produced in brain under ischemic conditions. Intracellular and extracellular pathways for adenosine formation have been described. Transgenic (Tg) mice with neuron-specific expression of hENT1 were developed (Parkinson et al., 2009 J. Neurochem. 109:562-572) to examine neuronal uptake and release of adenosine during ischemic conditions.
Objectives: The present study examined release of adenosine and inosine from primary cultures of cortical neurons from wild type (CD1) and hENT1 Tg mice under basal and excitotoxic conditions.
Methods: Primary neuronal cultures were incubated with ^{3}H-adenine to radiolabel intracellular ATP. Cells were then treated for 30 minutes (37 ºC) with buffer or N-methyl-D-aspartate (NMDA; 100 μM). The effects of dipyridamole (DPR; 30 μM), an inhibitor of ENT1 and ENT2, α,β-methylene ADP (AOPCP; 50 μM), an inhibitor of ecto 5'-nucleotidase, and S-(4-nitrobenzyl)-6-thioinosine (NBMPR; 100 nM), a selective ENT1 inhibitor, on adenosine and inosine production were assessed.
Results: NMDA significantly increased levels of both adenosine and inosine ($p < 0.05$); levels were significantly greater using hENT1 Tg neurons than using CD1 neurons ($p < 0.05$). DPR, but not AOPCP or NBMPR, significantly inhibited levels of both adenosine and inosine ($p < 0.05$).
Conclusions: Intracellular formation and transporter-mediated release of both adenosine and inosine occurs from neurons under ischemia-like conditions. This contrasts with findings from hippocampal slices treated with ischemic conditions, which exhibited transporter-mediated uptake of adenosine.

Disposition of Atorvastatin, Rosuvastatin and Simvastatin in Oatp1b2$^{-/-}$ Mice and Intraindividual Variability in Human Subjects (CSPT-2)

DeGorter MK[1], Urquhart BL[1], Tirona RG[1,2], Kim RB[1,2].
[1]Department of Physiology and Pharmacology and [2]Division of Clinical Pharmacology, Department of Medicine, The University of Western Ontario, London, Ontario, Canada.
Corresponding Author: marianne@degorter.ca
Conflict of Interest: None declared

Background: The HMG-CoA reductase inhibitors, or statins, are widely prescribed to reduce cardiovascular disease risk. There is considerable interindividual variation in statin exposure and response arising from variability in both transport and metabolism.
Objective: Our aim was to better understand the *in vivo* relevance of the organic anion-transporting polypeptide (OATP) 1B family to atorvastatin (ATV), rosuvastatin (RSV) and simvastatin (SVA) disposition in Oatp1b2$^{-/-}$ mice, and the role of metabolism *vs* transport by comparing ATV, RSV and SVA pharmacokinetics in healthy human subjects given all three statins.
Methods: Male Oatp1b2$^{-/-}$ and wild-type mice were dosed 1 mg/kg ATV, RSV or SVA IV, and liver and plasma concentrations measured 30 min later. Ten healthy subjects were administered a single oral dose of 20mg ATV, 20mg SVA and 10mg RSV in a cross-over study design. Statin acid concentration in plasma collected 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8 and 10 h post-dose was measured by LC-MS/MS.
Results: Liver-to-plasma ratios were significantly lower in Oatp1b2$^{-/-}$ *vs* wild-type mice for ATV ($p=0.002$) and RSV ($p=0.03$) but not SVA. In

humans, plasma exposure of ATV and SVA acid were significantly related ($p<0.05$), while RSV profile was not predictive of ATV or SVA acid exposure.
Conclusions: In mice, Oatp1b2 appears important for the hepatic uptake of ATV and RSV. In humans, ATV and SVA, which are subject to CYP3A metabolism and transport, appear to share common mechanisms of elimination, in contrast to RSV, which is not significantly metabolised but a substrate of multiple hepatic uptake and efflux transporters.

Validation of the Novel *in vitro* Platelet Toxicity Assay (*i*PTA) for the Diagnosis of Drug Hypersensitivity Reactions (CSPT-3)

Elzagallaai AA[1,2], Rieder MJ[2,3], Koren G[1,2,3].
[1]Ivey Chair in Molecular Toxicology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada; [2]Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada; [3]Department of Pediatrics, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada.
Corresponding Author: aelzagal@uwo.ca
Conflict of Interest: None declared

Background: Drug hypersensitivity reactions (DHRs) are rare but potentially fatal adverse events which occur in susceptible patients. The diagnosis and prediction of DHRs is difficult due to their variable clinical presentation and the overlap of symptoms with other clinical conditions. Systematic rechallenge, the gold standard for DHRs diagnosis, is not always ethical to perform due to possible serious reactions. Current *in vitro* tests including the lymphocyte toxicity assay (LTA) are cumbersome. We have recently developed a novel *in vitro* diagnostic test, the *in vitro* platelet toxicity assay (*i*PTA) for DHRs.
Objective: To validate the iPTA as a diagnostic test for DHRs.
Methods: Twenty-eight individuals (14 DHS-sulfa patients and 14 healthy controls) were recruited. Blood samples were obtained and both LTA and iPTA were performed independently. Results were then compared to determine the degree of agreement between the two diagnostic approaches.
Results: There was concentration-dependent toxicity in the cells of patients when incubated with the reactive hydroxylamine metabolite of sulfamethoxazole for both the LTA and iPTA ($p<0.05$) and toxicity was significantly greater for the cells of patients versus controls ($p<0.05$). The two tests had a high degree of agreement (correlation coefficient: $R^2 = 0.97$). It was very clear that the iPTA was more sensitive than the conventional LTA test in detecting the susceptibility of patient cells to *in vitro* toxicity.
Conclusion: The novel iPTA has considerable potential as an investigative tool for DHS as it is cheaper to perform and requires no special reagents that make it more suitable for clinical wider use.

Role of Efflux Transporter P-glycoprotein (MDR1) in Rivaroxaban Drug Disposition (CSPT-4)

Gong IY[1], Mansell SE[2], Kim RB[1,2,3].
[1]Department of Physiology and Pharmacology, 2Division of Clinical Pharmacology, Department of Medicine, 3Lawson Health Research Institute, University of Western Ontario, London ON, Canada.
Corresponding Author: ygong6@uwo.ca
Conflict of Interest: None declared

Background/Objective: Thromboembolic events resulting from blood clotting disorders are a significant source of mortality and morbidity. The novel direct factor 10a inhibitor, rivaroxaban, was recently approved for thromboembolism prophylaxis following total knee or hip replacement. However, rivaroxaban renal elimination is greater than glomerular filtration rate; thus, we hypothesized that the efflux drug transporter, P-glycoprotein (MDR1), is involved in rivaroxaban excretion and disposition.
Methods: The ability of MDR1 to mediate rivaroxaban transport *in vitro* was assessed in LL-CPK cells overexpressing MDR1 (LMDR1). To determine the *in vivo* relevance of MDR1 to rivaroxaban disposition, plasma and tissue concentrations were determined in *Mdr1a* deficient mice ($Mdr1a^{def}$) following oral administration.
Results: A markedly higher vectorial transport of rivaroxaban was observed in the basolateral to apical direction (B-A) compared to A-B in LMDR1 cells (efflux ratio 5.6, n=5). Additionally, a selective inhibitor of MDR1 (LY335979) abolished the B-A transport of rivaroxaban (efflux ratio 1.2, n=3). Following oral administration of rivaroxaban *in vivo*, plasma concentrations did not significantly differ between wild-type and $Mdr1a^{def}$ mice (n=6). Liver to plasma ratio of rivaroxaban concentration was significantly lower in $Mdr1a^{def}$ mice ($P<0.01$), while

kidney to plasma ratio was marginally higher compared to wild-type mice. Importantly, rivaroxaban brain concentrations did not differ, suggesting that other efflux transporters at the level of blood-brain barrier may be compensating for the absence of MDR1.
Conclusions: Overall, rivaroxaban appears to be a substrate MDR1 *in vitro*. However, further studies are required to elucidate additional efflux transporters involved in rivaroxaban disposition *in vivo*.

Limited Placental Transfer of 6-Mercaptopurine is Mediated by Tissue Binding and not Active Transport (CSPT-5)

Hutson JR[1,2], Lubetsky A[1], Walfisch A[1], Garcia-Bournissen F[1,2], Koren G[1,2].
[1]Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, Toronto, [2]Insitute of Medical Sciences, University of Toronto, Toronto.
Corresponding Author: j.hutson@utoronto.ca
Conflict of Interest: None for all authors

Introduction: The immunosuppressant azathioprine is increasingly used in pregnancy for the treatment of autoimmune diseases or for organ transplant patients. Several studies have demonstrated that azathioprine does not increase the risk for major fetal malformations. Azathioprine is rapidly metabolized to 6-mercaptopurine (6-MP) and the placenta is considered a relative barrier to 6-MP. Because 6-MP interferes with DNA synthesis, it is important to determine how the placenta restricts transfer in order to identify factors that could increase fetal exposure.
Objective: To determine if active drug transporters, tissue binding, or placental metabolism restrict 6-MP transfer.
Methods: Dual perfusion of a single human placental lobule *ex vivo* was utilized and 6-MP was added to the maternal circulation to determine transplacental kinetics. 6-MP was also introduced under equilibrative conditions to determine if 6-MP is actively effluxed into the maternal circulation. Metabolite formation was also measured during all perfusions.
Results: At a clinically relevant concentration (50ng/ml), 6-MP appeared in the fetal circulation after 30 minutes. After 180 minutes, the fetal:maternal 6-MP concentration ratio was 0.45±0.09 (n=3). After adding 6-MP to both the maternal and fetal circulations, the fetal:maternal concentration ratio was 1.205±0.177 (n=4) after 180 minutes. 6-methylmercaptopurine was the only metabolite detected and only in perfusions with 10-fold higher 6-MP concentrations.
Conclusions: Tissue binding to the placenta, together with a short half-life, limits placental transfer of 6-MP. Active transport does not play a major role, thus polymorphisms or drug interactions involving drug transport proteins are unlikely to leave a fetus more vulnerable to 6-MP exposure.

Incidence of Central Nervous System (CNS) Depression of Neonates Breastfed by Mothers Receiving Oxycodone for Postpartum Analgesia (CSPT-6)

Lam J[1,2], Kelly L[3], Ciszkowski C[3,4], Carleton BC[5,6], Hayden MR[5,7], Madadi P[2], Koren G[1,2,3,4]
[1]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Canada; [2]Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, Toronto, Canada; [3]Department of Physiology and Pharmacology, [4]Ivey Chair in Molecular Toxicology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Canada; [5]Child and Family Research Institute, Children's and Women's Health Centre of British Columbia; [6]Pharmaceutical Outcomes Programme, Children's and Women's Health Center of British Columbia, Vancouver, Canada; [7]Department of Medical Genetics, Center for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada.
Corresponding Author: jessica.lam@sickkids.ca
Conflict of Interest: None declared

Background: Oxycodone has recently replaced codeine for postpartum pain relief in some institutions. However, the neonatal safety of oxycodone during breastfeeding is unknown.
Objective: To quantify the incidence of neonatal CNS depression in oxycodone-medicated mothers compared to codeine, and acetaminophen-only group.
Methods: A retrospective study consisting of 3 cohorts in 533 breastfeeding mother-infant pairs exposed to oxycodone (n=139), codeine (n=210) or acetaminophen-only (n=184) was conducted. A standardized telephone questionnaire was administered to elucidate adverse maternal and neonatal events temporally related to either drug according to maternal self-reports.
Results: The incidence of neonatal CNS depression

for oxycodone was 20.1% (28/139) compared to 16.7% (35/210) for codeine [p>0.05, OR 0.79 95% CI 0.46-1.38] and 0.5% for acetaminophen (1/184) [p<0.0001, OR 46.16 95% CI 6.2-344.2]. Mothers of symptomatic neonates in the oxycodone and codeine cohorts took significantly higher doses of medication compared to mothers of asymptomatic infants in the same cohorts [oxycodone p=0.0005 (median 0.4 (0.03-4.06) vs. median 0.15 (0.02-2.25) mg/kg/day and codeine p<0.001 median 1.4 (0.7-10.5) vs. 0.9 (0.18-5.8) mg/kg/day]. There was significant concordance between neonatal and maternal CNS depression in both oxyodone and codeine groups [p=0.0006: OR 8.86, 95% CI 2.00-39.24, p<0.001: OR 21.1 95% CI 7.4-60.6 respectively].
Conclusion: Oxycodone is not a safer alternative than codeine in breastfed infants.

Effects of Chronic Renal Failure on Brain Drug Transporters in Rats (CSPT-7)

Naud J[1,2], Michaud J[1,2], Beauchemin S[1], Lefrancois S[1], Leblond FA[1], Pichette V[1,2].
[1]Centre de recherche de l'Hôpital Maisonneuve-Rosemont, Montréal, Québec; [2]Département de pharmacologie, Faculté de Médecine, Université de Montréal, Montréal, Québec.
Corresponding Author: judith.naud@umontreal.ca
Conflict of Interest: None declared

Background: Many studies demonstrated that chronic renal failure (CRF) significantly affects the expression and activity of intestinal, hepatic and renal drug transporters. These drug transporters are also expressed in brain cells and at the blood-brain-barrier (BBB) where they limit the entry and distribution of drugs in the brain. Perturbations in brain drug transporters equilibrium caused by CRF could lead to central toxicity of drugs.
Objective: To evaluate how CRF affects the expression and activity of drug transporters at the BBB and in the brain using nephrectomised rats.
Method: Protein and mRNA expression of influx transporters (organic-anion-transporting-polypeptide [Oatp], organic-anion-transporter [Oat]), and efflux transporters (p-glycoprotein [P-gp], multidrug-resistance-related-protein [MRP]) was measured in CRF and control rat brain biopsies. Intra-cerebral accumulation of radio-labelled benzylpenicillin (substrate of Oats and MRPs) and digoxine (Oatps, P-gp) was used to evaluate BBB permeability to drugs. Protein expression of transporters was evaluated in rat brain endothelial cells (RBEC) and astrocytes incubated with control and CRF rat serum.
Results: We demonstrated significant 30-50% decreases in protein and mRNA of MRP2-4, Oat3, Oatp2-3 and P-gp in CRF rat brain biopsies, astrocytes and RBEC. MRP5 was unchanged. We found a 30% decrease in BBB permeability of benzylpenicillin and no change in digoxine permeability. We hypothesize that similar reductions in the expression and activity of influx and efflux transporters prevented drug accumulation in the brain and that competition with accumulating endogenous organic anions explains the reduced permeability of benzylpenicillin.
Conclusion: Even with decreased drug transporters, BBB integrity seems to be conserved in CRF.

Repeated Ethanol Exposure of Fetal Sheep in Late Gestation and Fatty Acid Ethyl Esters in Meconium (CSPT-8)

Zelner I[1,2], Kenna K[3], Brien J[4], Bocking A[5], Harding R[6], Walker D[3], Koren G[1,2].
[1]Div. of Clinical Pharmacology & Toxicology, The Hospital for Sick Children, Toronto, ON, Canada; [2]Dept. of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada; [3]Dept. of Physiology, Monash University, Clayton, VIC, Australia; [4]Dept. of Pharmacology and Toxicology, Queen's University, Kingston, ON, Canada; [5]Dept. of Obstetrics and Gynaecology, University of Toronto, Toronto, ON, Canada; [6]Dept. of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia.
Corresponding Author: i.zelner@utoronto.ca
Conflict of Interest: None for all authors

Background: Meconium fatty acid ethyl esters (FAEEs) are established biomarkers of prenatal ethanol exposure. We hypothesized that meconium FAEEs content correlates with ethanol-induced fetal pathology, thus validating their use in identifying newborns at-risk for ethanol-induced disabilities.
Objective: To determine the relationship between various markers of fetal neuropathology and organ-system injury and FAEEs content in meconium of fetal sheep exposed to ethanol in late gestation.
Methods: From 95-133 days of gestational age (DGA; term ~147 days), chronically catheterized pregnant ewes received daily 1-hr infusions of either 0.75 g ethanol/kg (*n*=13) or saline (*n*=9). On 134 DGA, ewes and fetuses were euthanized and fetal

tissues, as well as meconium, were collected for analysis. Meconium FAEEs (palmitic, linoleic, oleic, and stearic) were quantified using headspace solid-phase microextraction and gas chromatography-mass spectrometry.

Results: Total FAEEs content was significantly higher in ethanol-exposed fetuses as compared with controls ($P<0.05$), with mean content in meconium of 0.174 nmol/g (range 0-0.788) for ethanol and 0.013 nmol/g (range 0-0.068) for controls. Ethyl stearate and ethyl palmitate were measurable only in ethanol-exposed fetuses; ethyl oleate was measurable in both ethanol and controls, but in higher amounts in ethanol-exposed fetuses; while ethyl linoleate was undetectable in both groups.

Conclusions: Ethanol exposure in late gestation resulted in elevated FAEEs in meconium of fetal sheep. Correlational analysis of meconium FAEEs content with post-mortem measures of neuropathology in immersion-fixed fetal brain slices and with fetal organ-system pathology will be conducted to determine whether increased FAEEs content is predictive of particular manifestations of ethanol teratogenicity.

CSPS Award Lectures / CC-CRS Travel Awards

Thursday AM

GlaxoSmithKline/CSPS Early Career Award

Platform of Biopharmacy: Injecting Quality into Drug Discovery

Grégoire Leclair, Professeur de technologie pharmaceutique, Faculté de pharmacie, Université de Montréal, Montréal, Quebec

[Abstract not available at time of printing]

Grégoire Leclair
Dr Leclair obtained his Bachelor of Pharmacy from Université de Montréal and his Pharmacist license from the Ordre des pharmaciens du Québec in 1996. He pursued his graduate studies at the same institution where he completed a M.Sc. and then a Ph.D. in Pharmaceutics. His work involved the synthesis of novel functionalized biopolymers, formulation of adhesive microparticles and creative modelling/simulation of the release kinetics from microparticles using cellular automaton. Upon completion in 2003, Dr Leclair was employed by Ratiopharm Canada as a Formulation Scientist. He contributed to the development of several generic solid oral dosage forms including product definition, excipient and process selection, formulation development, manufacturing and scale-up to 1/lOth production scale. He later joined Merck Frosst from 2004 to 2008 where he had numerous significant contributions particularly in the development of a nanomilling platform to support the lead optimization of inhalation drugs. Since 2008, he has been Assistant Professor at the Faculte de pharmacie of Université de Montréal. Over the last few years, he has set-up the basis of a strong academic research program aimed at novel formulation strategies to prepare drug nanoparticles for controlled release and targeted delivery applications.

CSPS Award of Leadership in Canadian Pharmaceutical Sciences

The Development of a Novel Oral Formulation of Amphotericin B for the Treatment of Systemic Fungal Infections and Visceral Leishmaniasis

Kishor M. Wasan, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada

Amphotericin B (AmB) is a parenterally administered broad-spectrum antifungal and leishmanicidal drug that has been on the market for over sixty years. Unfortunately, significant infusion-related side effects and renal toxicity often accompany treatment, limiting its clinical applications. Lipid-based formulations have somewhat ameliorated the associated toxicity, but the increased cost of formulations restricts widespread use. AmB is amphipathic and exhibits low solubility and permeability, resulting in negligible absorption when administered orally. Advances in drug delivery systems have overcome some of the solubility issues that prevent oral bioavailability and new formulations are currently in development. The existence of an effective, safe and inexpensive oral formulation of amphotericin B would have significant applications for the treatment of disseminated fungal infections and would dramatically expand access to treatment of visceral leishmaniasis by introducing a readily available highly tolerated oral formulation of a drug with known efficacy.

Kishor M. Wasan
Dr. Kishor M. Wasan is a Distinguished University Scholar Professor at the University of British Columbia in Vancouver, BC, Canada. In the 15 years that Dr. Wasan has been an independent researcher at UBC, he has published over 180 peer-reviewed articles and 220 abstracts in the area of lipid-based drug delivery and lipoprotein-drug interactions. His work was highlighted in the

January 2008 Issue of Nature Reviews, Drug Discovery.

Dr. Wasan was one of the recipients of the 1993 American Association of Pharmaceutical Scientists (AAPS) Graduate Student Awards for Excellence in Graduate Research in Drug Delivery, the 2001 AAPS New Investigator Award/Grant in Pharmaceutics and Pharmaceutics Technologies, the 2002 Association of Faculties of Pharmacy of Canada New Investigator Research Award and recently was named an AAPS fellow in 2006. In addition, Dr. Wasan was awarded a Canadian Institutes of Health Research University-Industry Research Chair in Pharmaceutical Development (2003-2008), was named a University Distinguished Scholar in April 2004 received the 2007 AAPS Award for Outstanding Research in Lipid-Based Drug Delivery and the 2008 AFPC-Pfizer Research Career Award. In April 2009 Dr. Wasan was named CIHR/iCo Therapeutics Research Chair in Drug Delivery for Neglected Global Diseases. Currently Dr. Wasan's research is supported by several grants from The Canadian Institutes of Health Research, several pharmaceutical companies and the National Cancer Institute of Canada-Clinical Trials Group. In addition, Dr. Wasan is one of the founding members of the University of British Columbia's Neglected Global Diseases Initiative. To date the initiative has raised over 1.2 million dollars towards new therapeutics for neglected diseases.

Plenary Session 2

Thursday AM

First-in-Man: From Laboratory to Clinic of Biotechnology Entities

Helen Loughrey, Director, Immunology and Formulation Development, Theratechnologies Inc., Montreal

[Abstract not available at time of publication]

Helen Loughrey

Helen Loughrey completed her BA in Biochemistry at Trinity College, Dublin, Ireland, and her Ph.D. in Biochemistry at the University of British Columbia in liposome based drug delivery. Her experience in peptides started as a collaborative study with the Irish drug delivery company, ELAN in 1995. She worked at RTP-Pharma, Montreal on oral and IV delivery of peptides and sparingly soluble small molecules, using particle and micro-emulsion-based delivery systems. Prior to joining Theratechnologies Inc, Montreal, she was Director, Target Validation at Signalgene, Montreal. She has been at Theratechnologies Inc, Montreal, Canada, a peptide-focused company, since 2003 where she is presently Director, Immunology and Formulation Development. Over the years, among her responsibilities were to develop immunogenicity and biomarker analysis strategies supporting pivotal Pre-clinical and Clinical studies, new formulations and cell-based bioassays in support of Pharmaceutical Development and manage Pre-clinical drug delivery feasibility studies.

Thursday AM - Track 2

Bioactive Delivery 1: Nucleic Acid Therapies

Progress in the Development of Delivery Systems for siRNA

Mark A. Tracy, Senior Director, Pharmaceutical Operations, Alnylam Pharmaceuticals Inc., Cambridge, Massachusetts, USA

The development of approaches for delivering siRNA to desired cells *in vivo* is essential to realize the full potential of this class of molecules as therapeutics. Significant progress has been made over the last couple of years in advancing the science of siRNA delivery and in translating this knowledge to the clinic. As a result, multiple delivery approaches have now been used in the clinic for both local (or direct) and systemic delivery offering the potential to treat important diseases including RSV, liver cancer and TTR amyloidosis. This presentation will discuss the progress in developing delivery systems for siRNA with a focus on delivery by lipid nanoparticles (LNPs). Lipid nanoparticle delivery systems represent one of the few systemically administered delivery systems for siRNA that have entered the clinic. This presentation will provide a review of recent data supporting the advancement of a lipid particle formulation to the clinic and will discuss advances in research offering the potential for improved formulations in the near future. In particular, the discovery of new lipids has resulted in the improvement in potency of next generation formulations of up to 2 orders of magnitude for some liver targets. Also, improvements in the understanding of key formulation variables and mechanisms of cellular uptake of lipid nanoparticles (LNPs) may result in delivery to a greater number of locations or cell types within the body. These results support the potential of RNAi therapeutics as a new class of pharmaceuticals for the treatment of a variety of important diseases.

Mark A. Tracy

Dr. Tracy is Senior Director of Pharmaceutical Operations at Alnylam, Inc. He leads planning, technology development, and alliance management activities in developing siRNA delivery systems and translating them from research to the clinic. Most recently, he played a key role in advancing a novel systemically delivered lipid-based siRNA formulation to an IND filing, the first dual targeting RNAi therapeutic to advance to this stage of development. Previously, Dr. Tracy was Director of Formulation Development at Alkermes, Inc. where he and his group were responsible for the development of inhaled and injectable formulations of biologics and small molecules. He was a member of the team that developed and scaled-up the first sustained delivery system for proteins approved by the FDA and commercialized and contributed to the development of Risperdal® Consta and Vivitrol®, two small molecule containing microsphere sustained release formulations on the market. Dr. Tracy also contributed to the development of new inhalation technologies. He led project teams and various collaborations from feasibility through clinical testing. Dr. Tracy received his Ph.D. in Chemistry from Stanford University. He holds an M.S. in Chemical Engineering from Stanford and a B.S. summa cum laude in Chemical Engineering from the University of Illinois. Dr. Tracy has published journal articles and patents in the areas of drug delivery, pharmaceutics, polymers and colloids, and proteins and peptides. He is an adjunct associate professor in the Department of Pharmaceutical Sciences at Northeastern University and a lecturer at Brown University, and previously Harvard and the Massachusetts College of Pharmacy. He is President of the Controlled Release Society (CRS) in 2010-11 and previously served on the CRS Board of Scientific Advisors. He is a Fellow of AIMBE. He is a member of AIChE, ACS, AAPS, PDA, and AAAS.

Arabinose Modified Antisense and siRNAs: Biological Applications, Structural Considerations, and Delivery Strategies

Masad Damha, Professor, McGill University, Montreal, QC

Harnessing RNA interference (RNAi) for therapeutic gene silencing presents an attractive alternative to small molecule approaches. Unfortunately, *in vivo* gene silencing in a therapeutic setting has proven difficult. A wide array of chemical modifications has been developed to improve the therapeutic potential of siRNAs. We will discuss chimeric chemically modified siRNAs, combining a DNA analogue (namely 2'-fluoroarabinonucleic acids: 2'F-ANA) with RNA analogues. The described modifications allow for tuning of duplex thermodynamics, reduced immunostimulation, and increased nuclease resistance without impairment of gene silencing activity against reporter constructs and endogenous genes. This siRNA modification approach benefits from retention of an A-form dsRNA-like helical conformation. Structural aspects of modified siRNAs will be presented, and applications of modified siRNAs and antisense oligonucleotides (AONs) towards novel endogenous targets will be described, with a focus on ongoing anticancer applications.

To date, a means for effective delivery of both unmodified and modified AONs and siRNAs to target cells and tissues presents a major challenge impeding the development of therapeutic oligonucleotides. To this end, we will also discuss early advances towards new delivery strategies for modified siRNAs and AONs, with and without delivery vehicles to facilitate cell uptake.

Masad Damha

Dr. Masad J. Damha was appointed Associate Vice-Principal (Research and International Relations) of McGill University effective November 1, 2010. Born and raised in Managua, Nicaragua, Dr. Damha immigrated to Canada in 1978. He received his B.Sc. ('83 – Honours) and Ph.D ('88) from McGill University. In 1987, he became an Assistant Professor at Erindale College, University of Toronto. He returned to his Alma Mater in 1992 where, as James McGill Professor of Chemistry, he works in the field of bioorganic and biomedicinal nucleic acid chemistry. Dr. Damha has published over 135 peer reviewed scientific articles and holds several patents worldwide in the RNA therapeutics field. In 1999, Dr. Damha co-founded Anagenis, Inc., a McGill's spin off company with proprietary gene silencing technologies. He has received several awards for his research including the Merck-Frosst Centre for Therapeutic Research Award (CSC, 1999), Bernard Belleau Award (2007), the Fessenden Professorship in Science Innovation (2010), and the David Thompson Award in Graduate Supervision and Teaching (2010). He is a Fellow of the Chemical Institute of Canada, and an Honorary Member of the International Society of Nucleosides, Nucleotides & Nucleic Acids. Dr. Damha also serves on the Board of Directors of the Oligonucleotide Therapeutic Society.

Development of Lipid-Nucleic Acid Particles for RNAi

James Heyes, Director, Formulation Chemistry, Tekmira Pharmaceuticals, Burnaby, BC

LNP products containing siRNA are currently being evaluated in several clinical and preclinical studies. This presentation will examine the design and development of these lipidic systems. Recent progress in lipid chemistry and formulation strategies to expand the therapeutic utility of this platform technology will be discussed. The presentation will also provide information on the technical aspects of scale up, manufacturing and testing of these systems to ensure reproducibility and robustness.

James Heyes

James Heyes joined Tekmira in 2008 following the merger between Tekmira and Protiva Biotherapeutics Inc. A graduate of the University of Manchester in England, James received his PhD from the Institute of Cancer Research in London, where he studied the design of cationic lipids and the use of peptides as targeting ligands for nucleic acid delivery. James joined Protiva in 2001, shortly after the company was founded, and was a key contributor to the development of Protiva's Lipid Nanoparticle (LNP) technology. James' work enabled Protiva's Pro-1 clinical trial, the first systemic administration of plasmid DNA in human subjects. James is currently the Director of Tekmira's Formulation Chemistry group where he is responsible for the design of novel LNP components and siRNA formulations (three of which have entered the clinic), targeted delivery systems and

expansion of the LNP technology to enable alternative routes of formulation administration.

Realizing the Potential of siRNA Therapeutics by Delivery with Lipophilic Polymeric Carriers

Hasan Uludağ, Hamidreza M. Aliabadi, Remant K.C., Breanne Landry, Department of Chemical & Materials Engineering, University of Alberta, Edmonton, Alberta.

Targeting specific molecular changes causing cancer is increasing becoming feasible with the development of designer therapeutic agents. This goal is most readily achieved by using siRNA-based therapeutics, which are capable of selectively down-regulating expression of target molecules causing aberrant changes in normal cells. However, a suitable delivery system needs to be employed to deliver siRNA intracellularly, since the extracellular siRNA is neither stable nor capable of cellular entry. We at the University of Alberta are actively designing and engineering polymer-based nucleic acid carriers to realize this vision. Amphiphilic polymers with the appropriate balance of lipophilic and cationic moieties are created in order to deliver siRNA to a wide range of human cells. We have shown that several types of cationic polymers and lipophilic substituents could be effectively combined to act as siRNA carriers. This presentation will focus on the structure-function relations for several classes of lipophilic polymers. Physicochemical properties of siRNA complexes with the carriers will be presented and their effectiveness in melanoma [*Alshamsan,* et al, Biomaterials (2010) 31: 1420-1428], breast cancer [*Abbasi et al.,* Cancer (2010) 116: 5544-5554] and leukemic cells (unpublished) will be summarized. By using the developed carriers, the siRNA molecules were shown to act on their own to curb uncontrolled proliferation of cancerous cells, or to augment the traditional drug therapy by sensitizing the transformed cells to drug action. We will emphasize the structural features of the lipophilic polymers that are critical for siRNA delivery in these modes of therapeutic actions. Limitations of current systems will be highlighted with the expectation of improved design in the future. Careful design of siRNA carriers along with effective siRNAs against appropriate targets is likely to form the foundation of next-generation cancer drugs.

Hasan Uludağ

Hasan Uludağ, a native of Turkish Republic of Northern Cyprus, obtained dual B.Sc. degrees in Biomedical Engineering and Biology from the Brown University (Providence, RI, USA) in 1989. He then completed his Ph.D. degree in 1993 from the Department of Chemical Engineering & Applied Chemistry at University of Toronto (Toronto, ON, Canada). Dr. Uludağ spent four years at the Genetics Institute Inc., participating as a scientist on the development of a tissue engineered device intended for bone regeneration and repair. He subsequently joined the U. of Alberta, holding joint appointments at the Departments of Chemical & Materials Engineering, and Biomedical Engineering, Department of Dentistry and the Faculty of Pharmacy & Pharmaceutical Sciences. Dr. Uludağ is currently directing interdisciplinary research programs on novel approaches to bone regeneration and non-viral delivery systems for gene-based therapeutics. The research activity is focussed on development of advanced materials for functional delivery of a wide range of therapeutic agents, including small molecular entities, peptides and proteins, DNA and siRNA. Dr. Uludağ published >100 peer-reviewed papers, participated in the training and development of >70 research personnel, and is actively engaged in the worldwide biomaterials community.

Thursday AM - Track 3/4

Polymolecular Drug Discovery

SPONSORED BY: AFEXA LIFE SCIENCES INC.

CVT-002: An Immune Modulator that Enhances Anti-Viral Effects

Christine Lutsiak, Afexa Life Sciences Inc.

[Abstract not available at time of publication]

Shelly McNeil

[Abstract & title not available at time of publication]

The Role of CVT-E002 (*Panax Quinquefolius*) in Immunoenhancement and Cancer Abatement

Sandra C. Miller, Professor, Department of Anatomy & Cell Biology, McGill University, Montreal, Canada

Cells belonging to the innate immune system are called natural killer (NK) cells and one of their main roles is tumor immunosurveillance. Over 2 decades, we have succeeded in enhancing quantitatively, these cells in mice, *in vivo*, by means of drugs, cytokines and in recent years, phytoceuticals such as CVT-E002, a proprietary extract of North American ginseng (Afexa Life Sciences, Inc., Edmonton, AB, Canada). We found that in adult mice bearing leukemia, given daily CVT-E002, via the chow, their tumors progressively decreased, their life span was significantly extended, and their NK cells were quantitatively augmented in the bone marrow (NK cell generating site), and the spleen (organ of mature NK cell residence and site of tumor cytolysis). However, dosage was critical in producing these ameliorative effects. Following this, pre-weaned mice (age: 7d) were injected with leukemia cells, and subsequently injected daily for 14 days with CVT-E002, in 6 dosing groups. Again, in an exquisitely dose-dependent manner, the extract significantly extended the life span of these infant, leukemic mice. In another study, we assessed the short and long term potential of CVT-E002 (consumed daily via the chow) to enhance NK cells in *normal*, young adult mice (age: 2 mo.), and in *normal*, juvenile mice (age: 1 mo.), the latter given the extract in the chow beginning at weaning. Both young adult and juvenile mice were fed daily CVT-E002 for 1 mo., followed by 2 mo. consuming untreated chow, i.e., chow from which CVT-E002 was withdrawn. In both the young adult and juvenile mice, NK cells, even 2 mo after withdrawing CVT-E002, were quantitatively increased, implying a prophylactic influence of this extract on these tumour-combatting cells. Finally, we assessed the effect of 14 daily injections of CVT-E002 on *normal*, infant, suckling mice, from age 7 days until age 21 days. The data revealed that at 21 days, NK cells in the bone marrow and spleen were significantly higher than control, sham injected infants. However, even more potentially significant was the fact that although CVT-E002 was provided to these infants only during suckling, upon reaching adulthood NK cells were still quantitatively, significantly elevated over controls. In summary, CVT-E002 appears to have both therapeutic and prophylactic effects, the latter continuing even when the extract is no longer present.

Sandra C. Miller

Dr. Miller (B.Sc., Sir George Williams University, Montreal; M.Sc. and Ph.D., McGill University, Montreal, Canada; (Post-doctoral Fellow, Baylor College of Medicine, Houston, Texas), is a professor in the Department of Anatomy & Cell Biology, Faculty of Medicine at McGill University.

Her research interests include tracing the development, function and immuno-enhancement potential of specific cells involved in anti-tumor immunity, i.e., natural killer (NK) cells. She has

published 65 peer-reviewed articles as primary author, has been an invited speaker at several international and national conferences over the past 20 years and has guided, as sole supervisor, the research of Ph.D. and M.Sc. students, as well as that of 40 B.Sc. students.

Dr. Miller is heavily involved in the teaching of medical students, including medical school curriculum development. She has been a member, and/or chairperson of numerous committees at the Departmental, Faculty and University level and has contributed to the development of 2 major text books of anatomy, used the world over by medical students and others involved in health care occupations.

She has been recognized for her numerous academic and scientific achievements, by several awards, i.e., the JCB Grant Senior Scientist Award (2008) of the Canadian Association for Anatomy, Neurobiology & Cell Biology; the Canadian Association of Medical Educators Certificate of Merit Award (2006); Faculty Honours List for Educational Excellence (1999); and The Murray L. Barr Junior Scientist Award of the Canadian Association of Anatomists (1985).

Finally, Dr. Miller regularly reviews grant applications for national and international agencies as well as manuscripts for peer-reviewed scientific journals. She holds membership in 6 learned societies and has contributed, by invitation, to several health and cancer magazines aimed at the lay audience in both Canada and the USA.

A New Therapeutic Category of Polymolecular Botanical Drugs: A Case Study of CVT-E002 Potential in Chronic Lymphocytic Leukemia

Sharla Sutherland, Christine Lutsiak, Tara Lysechko, Erin MacKenzie, Lei Ling, Jacqueline J. Shan, Afexa Life Sciences Inc.

In the relatively recent history of modern pharmaceutical development, botanical substances have not been fully utilized as potential therapeutic agents. A new regulatory pathway has been implemented in the U.S. to enable the development of novel botanical drugs. These products must meet the same standards as single chemical entities, but due to the safe and extensive history of human use, accelerated development may be possible. Afexa's unique model involves using a rigorous drug development approach to develop safe natural ingredients into polymolecular botanical drug products with therapeutic claims based on the strongest possible evidence. This approach was utilized to develop CVT-E002™, a patented proprietary polysaccharide extract of *Panax quinquefolius* that has been shown to work as an immunomodulator. In Canada, a product licence was issued to the CVT-E002™-containing product, COLD-FX®, with the therapeutic claim 'Helps reduce the frequency, severity, and duration of cold and flu symptoms by boosting the immune system' based on randomized, double-blind, placebo-controlled trials. The ability of CVT-E002™ to reduce Acute Respiratory Illness (ARI) in an immune-suppressed population – Chronic Lymphocytic Leukemia (CLL) – was recently investigated in a Wake Forest University led and U.S. National Cancer Institute-approved multi-centre double-blind, placebo-controlled, randomized trial. The study involved 293 subjects with early-stage, untreated CLL randomized to receive either CVT-E002™ (400 mg/d) or placebo over the 2008/2009 cold and flu season. The study looked at ARI, antibiotic utilization, and various immunological and safety assessments. Preliminary data showed a reduction in the incidence of moderate-severe ARI symptoms, including a statistically significant 57% relative risk reduction in sore throat ($p = 0.004$). In addition, a trend of reduced incidence of moderate-severe ARI was observed, with 32% in the CVT-E002 group vs 39% in the placebo group (diff -7%, 95% C.I. -18%, 4%). There was no significant difference shown for patients with mild ARI symptoms, average number of ARI days or antibiotic use. CVT-E002 also significantly reduced the relative risk of Serious Adverse Events (SAEs, grade 3+ toxicities; $p = 0.02$). The lack of statistical significance for some observed trends may be due to insufficient dose, as well as the higher level of variability in ARI symptoms. Taken together, the data suggest that further studies in CLL are warranted and that CVT-E002™ may be one potential member of an emerging new category of polymolecular botanical drugs. The U.S. botanical drug regulatory path represents a unique opportunity for accelerated development of a highly innovative evidence-based and potentially safer new class of therapeutics.

Thursday PM - Track 1

Pharmacogenomics/Personalized Medicine

Pharmacogenomics in the Treatment of Breast Cancer

David A Flockhart, MD, PhD., Indiana Institute for Personalized Medicine, Indiana University School of Medicine

Both the risk of breast cancer and it's mortality are increasing worldwide. The treatment of breast cancer has improved markedly over the last 10 years with increasingly individualized approaches that have paved the way towards a more personalized treatment of many other malignancies. These include the targeting of endocrine therapy towards tumors that have estrogen or progesterone receptors, of the ant-HER2 antibody trastuzamab (Herceptin™) to tumors that overexpress the Her2, and the targeting of chemotherapy to patients with specific multigene RNA expression profiles such as the Oncotype Dx™ and Mammaprint™ tests.

Germline approaches have not been used to study efficacy of anti-cancer therapy until recently. Concentrations of the active metabolite of endoxifen clearly associate with CYP2D6 genotype, but studies conducted in the prevention, adjuvant and metastatic settings are not clear as to whether CYP2D6 poor metabolizer genotype confers altered response to tamoxifen treatment. Patients who are poor metabolizers of tamoxifen appear to tolerate the drug better and to drop out of trials and from therapy at a notably lower rate. Germline genetic variability in the VEGF receptor may also be important, since such variants were associated with outcomes, and can identify 2 populations: a group that experienced overall survival benefits not different from placebo, and a group that survived on average a year longer. Collectively these data suggest that assessment of germline genomic variability may be able to refine the targeting of patients with breast cancer to maximize efficacy, and reduce toxicity.

Pharmacogenetics for Predictive and Personalized Medicine in Post-Genomic Era: A Long Way Ahead

Candan Hızel, Ph.D C2H-Vichy Genomics, Vichy, France, Pharmacogenetics Consultants, Yeditepe University & Marmara University Faculty of Pharmacy Pharmacogenetics and Drug Safety Unit, Istanbul, Turkey

The era of post-genomic medicine arrived with the completion of the Human Genome Project in 2003, exactly 50 years after the discovery of DNA by Watson and Crick which means integration of genetic knowledge in our everyday life.Tremendous progress has been done during last several years in modern medicine due to rapid development of molecular medicine specifically genetics and also as informatics which has accelerated the use of Predictive and Personalized Medicine in routine medical and pharmacy practise for more efficient treatment of individuals. However, for most clinicians, the post-genomic era has not yet arrived. Today, the physicians still have to optimize a dosage regimen for an individual patient by "trail-and error" method. This kind of blind approach may cause many important adverse drug reactions, hospitalizing problem and many avoidable deaths. In addition, inefficient treatment cost a lot of money. The concept of predictive and personalized medicine is a lifelong and pharmacogenetics, the study of how genetic differences influence the variability in patients' responses to drugs, forms the cornerstone of predictive and personalized medicine. The most important barriers delaying clinical uptake and application of pharmacogenomics is lack of knowledge and insufficient education of health professionals regarding pharmacogenetics rather than technical issue. Moreover, there is a lack of qualification of information concerning to pharmacogenetic testing results, the pharmacogenetic testing results without precise

personalized interpretation regarding to patient's peculiarities, such as his/her life style factors like smoking or nutritional status and his/her clinical data could not be useful for medical professionals and patients.

We are in post-genomic era, it is now time… don't miss out on this opportunity, make your genes work for you and take control of your health!

Power and Limits of Large Scale Genomics as a Tool for Personalized Medicine

Pavel Hamet, Canada Research Chair in Predictive Genomics, CHUM, Université de Montréal

Large scale genomics is currently represented by GWAS, Genome Wide Association Studies in population- or case-control- based cohorts that include thousands of subjects. Currently 2 million single nucleotide polymorphisms are interrogated for their associations with quantitative traits (blood pressure, BMI, microalbuminuria), biological and clinical markers (cholesterol, HDL, CRP), outcomes (myocardial infarction, stroke, renal failure, sudden death) or treatment responsiveness in a large number of disease entities. It is progressively realized that the most significantly (genome wide) associated SNPs, determine only a small percentage of the trait variance, i.e. contributing only little to overall genetic determination, comparatively to the evidence obtained from family history or familial aggregation of these diseases. Our current understanding is inclined towards a belief that many gene variants have to be involved, each one having relatively minute contribution on its own. Gene x gene and gene x environment interactions are only partially taken into account, mainly due to the complexity of their analysis. Aging and environmental factors provoke relatively permanent modifications of the DNA molecule, such as telomere shortening and DNA methylation, leading to modulation of gene expression. An additional problem is the frequent gap between powerful and accurate genotyping technology that is not always accompanied by adequate in depth phenotyping efforts: thus systolic blood pressure level recorded under medication i.e. environmentally modified, can completely obscure the genomic contribution to the increases in blood pressure. Only a full integration of all these factors will allow the use of this new knowledge in the path to Personalized Medicine, for its evaluation of its eventual clinical utility.

Pavel Hamet

Dr. Hamet is the Canada Research Chair in Predictive Genomics. He is Professor of Medicine at Université de Montréal, Adjunct Professor of Experimental Medicine at McGill University, and Visiting Professor at the First Faculty of Medicine at Charles University, Prague, Czech Republic. He is currently Chief of Gene Medicine Services, member of the Endocrinology Service, and Director of the Laboratory of Molecular Medicine at the CHUM. He is also Director of Medical affairs and principal Investigator for CART@GENE project. He serves on many national and international boards including the Institute of Circulatory and Respiratory Health of the Canadian Institutes of Health Research (CIHR) and he is President-elect of the International Society of Pathophysiology. He is also President and Chief Scientific Officer of Medpharmgene as well as President and Chief Executive Officer of Prognomix. He has received many honours, including the prestigious Wilder Penfield Award in 2001. In 2008, Dr Hamet was named as an Officer of the Ordre national du Québec and received the Okamoto Award by the Japan Vascular Disease Research Foundation. Since 2010, he is Associate Editor of Journal of Hypertension.

Thursday PM - Track 2

Bioactive Delivery 2: Orthopedics

SPONSORED BY: AMGEN

Bone Targeting Pro-drugs: Strategies for Treatment of Osteoporosis and Other Bone Diseases

Robert Young, Department of Chemistry, Simon Fraser University, Burnaby, BC

Bone is a unique and dynamic tissue that is continuously renewed through life and which requires specific and targeted efforts to develop needed therapies and technologies to treat bone diseases and to promote renewal and repair. Many potential bone drug targets are also expressed widely thoughout the body and thus bone drugs may elicit undesired systemic side effects. It is also important to develop strategies for bone therapies that provide active drug at the site of action on a sustained basis and allow infrequent dosing. One useful method to address these needs is to use bone-targeting pro-drugs where the drug to be delivered is conjugated to a bisphosphonate moiety. Bisphosphonates bind essentially irreversibly to bone and are in themselves a useful class of drugs used to treat osteoporosis (by inhibiting resorption) and bone cancer. Bisphosphonate conjugated pro-drugs have been used in the past but have generally used the BP as a carrier and not an active partner in the treatment. We have designed dual-action prodrugs, wherein an active drug is conjugated to a bisphosphonate through a linker that is enzymatically hydrolysed in situ in the bone to yield both the active drug and the active bisphosphonate over a sustained time period. This technology has been used to deliver a proven bone growth stimulant (a prostaglandin EP4 receptor agonist) and a potent bone resorption inhibiting bisphosphonate (alendronic acid) and a variety of linkers have been designed to release active constituents over periods of days or weeks..

Robert Young

Robert Young earned a B.Sc. from the University of Victoria in 1967, a Ph.D. from the University of British Columbia in 1971 and continued with postdoctoral studies at the Imperial College of Science and Technology, the University of Adelaide and the University of British Columbia between 1971 and 1976. From 1976 to 1977, he was a Research Associate at the Institut de Chimie des Substances Naturelles in Gif-sur-Yvette, France, and from 1977 until 2006 he worked in various capacities with Merck Frosst Canada & Co and including assignments as Research Site Head at Merck Frosst (1998-1999) and Site Head at the Merck Research Laboratories Neurosciences Research Centre at Terlings Park, UK (2004-2005). He was Vice-President and Head of Medicinal Chemistry at the Merck Frosst Centre for Therapeutic Research from 1993 until taking early retirement in 2006. He is now Professor of Chemistry and Merck Frosst-B.C. Leadership Chair in Pharmaceutical Genomics and Drug Discovery in the Chemistry Department at Simon Fraser University. He is also chair of the Division of Medicinal Chemistry of the Center for Drug Research and Development (CDRD) in British Columbia and consults for a number of Pharmaceutical companies through his own company, Promorpheus Consultants Inc.

Dr. Young's career has focused on the design and synthesis of novel drugs for asthma, inflammation, osteoporosis and related diseases and he is most noted for his part in the discovery of the asthma drug, Singulair™ as well as the antilipidemic drug, Tredaptive™ and the anti-inflammatory drugs Arcoxia™ and Precicox™. His work on osteoporosis research led to the discovery of an investigational drug, odanicatib currently in final stages of clinical development. Dr. Young is the author of more than 190 publications, review articles and patents. His

current research is focused on the design and use of novel pharmacological probes and proof of concept molecules for the discovery and validation of new drug targets and the design of new drugs to treat bacterial infections, cancer, and inflammatory and bone diseases.

Dr. Young is a member of the Order of Canada and a Fellow of the Royal Society of Canada, Fellow of the Chemical Institute of Canada and is currently President Elect and Fellow of the Canadian Society for Pharmaceutical Sciences. His academic and professional honours include a National Merit Award from the Ottawa Life Sciences Council and a Heroes of Chemistry Award from the American Chemical Society. He was appointed a member of the Natural Sciences and Engineering Research Council of Canada and was recently recipient of the Award of Leadership in Pharmaceutical Sciences from the Canadian Society for Pharmaceutical Sciences, the first Genome BC Leadership Award and the Distinguished Alumni Award from the University of Victoria.

Materiomics – Dealing with Complexity in Tissue Engineering

Clemens A. Van Blitterswijk, MIRA-Institute for Biomedical Technology and Technical Medicine, Department of Tissue Regeneration, University of Twente, Enschede, The Netherlands

As the human body holds some 200 cell types that synthesize a multitude of both soluble and solid actives in addition to a variety of components that provide various means of mechanical support, it will be clear that extremely complex interactions stand at the basis of the proper functioning of all tissues.

With the increase of complexity, certainly when this is associated with a, at best, only partial understanding of the underlying mechanisms, special strategies need to be applied to unravel or direct processes that result from such complex interactions. Rather than striving for a full understanding of the underlying mechanisms upon which to base ones actions, it might be more productive to rapidly screen a multitude of approaches and select the one with the most optimal result. Surprisingly, in tissue engineering this approach is still largely unexplored. In this presentation, apart from a selective overview of the current state of high throughput research in tissue engineering, we will discuss the production of large libraries of material geometries that will allow us to screen thousands to millions of substrates. We propose the name *MATERIOMICS* for the discipline of high throughput methods in biomaterials and tissue engineering science. In addition, we will discuss novel approaches to screen soluble compounds to be used in tissue regeneration in a high-throughput fashion.

Clemens A. van Blitterswijk

Dr. Clemens A. van Blitterswijk graduated as cell biologist at Leiden University in 1982. He defended his PhD thesis in 1985 at the same university, on artificial ceramic middle ear implants, for which he was awarded the Jean Leray award, the Marie Parijs award and the Klein award in the following years. He continued his research on bone and cartilage replacement primarily, with extensions to cartilage and skin substitutions.

Today most of his research deals with tissue engineering and regenerative medicine. Dr. van Blitterswijk has authored and co-authored ca. 224 scientific papers (peer reviewed, Pubmed), guided 45 PhD students as promotor or co-promotor, is inventor of numerous patents and frequently acts as invited speaker or chairman at international conferences. For his more recent work he received the George Winter award of the European society for Biomaterials and was appointed Fellow of Biomaterials Science and Engineering by the international liaison committee.

He acts and has acted on numerous advisory organs relating directly to life- and material sciences or to the economic applications thereof and held positions such as chairman of the Dutch society for Biomaterials and treasurer of the European Society for Biomaterials. Current functions comprise:

Chairman of the Netherlands Forum for Regenerative Medicine, Council Member of the European Chapter of TERMIS (Tissue Engineering and Regenerative Medicine International Society), Chairman of TeRM (Translational Regenerative Medicine, SmartMix), Member of the Advisory Board of the Netherlands Technology Foundation, Member of the Netherlands Academy for Technology and Innovation.

During his career Dr. van Blitterswijk has co-founded multiple biomedical companies and held several functions in these organizations. He acted as CEO of IsoTis (a public life sciences company in the Netherlands) from 1996 to 2002. In his career he raised over 140 Million Euros in funding through equity and/or grants. Resulting from his work 10 implant technologies were brought into clinical

evaluation in humans. Today Dr. van Blitterswijk has an appointment at the University of Twente in the Netherlands where, as Professor of Tissue Regeneration, he heads a team of PhD students active in the field of tissue engineering and Regenerative Medicine. Recently he has accepted the function of scientific director of the Institute of Biomedical Technology and Technical Medicine.

Sclerostin Antibody: A Potential New Therapeutic Approach for Osteoporosis and Fracture Healing

Xiaodong Li, Amgen Inc., Thousand Oaks, CA

Genetic mapping and phenotypic studies of the rare, high bone mass human genetic disease, sclerosteosis led to the discovery of sclerostin and its characterization as a key inhibitor of bone formation. Similar to sclerostin inactivation in humans, mice with a targeted deletion of the sclerostin gene (*SOST* knockout mice) have high bone mass, demonstrating evolutionary conservation of sclerostin's function as a negative regulator of bone formation. In preclinical studies with sclerostin neutralizing monoclonal antibodies, significant increases in bone formation, bone mass and bone strength have been achieved in several bone-related disease models including a rat model of postmenopausal osteoporosis (PMO). Sclerostin antibody anabolism produces bone volume increases in both the cortical and trabecular bone compartments, with robust increases in bone formation being observed on trabecular, periosteal and endocortical bone surfaces. Furthermore, sclerostin antibody increased bone formation and bone strength at fracture site in several animal models of bone healing. In a Phase 1 clinical study, single-dose administration of sclerostin antibody in healthy men and postmenopausal women was found to increase several markers of bone formation, decrease a serum marker of bone resorption and increase bone mineral density. These results suggest that sclerostin inhibition by a monoclonal antibody represents a potential new therapeutic approach for osteoporosis and fracture healing.

Xiaodong Li

Dr. Xiaodong Li is a Principal Scientist in the Bone Biology group at Amgen Inc and supervises a group focused on *in vivo* pharmacology. He has been actively involved in the publication of pharmacology studies for Amgen's sclerostin and OPG programs. He has authored or co-authored more than 30 peer-reviewed articles, including key, proof-of-concept publications demonstrating the powerful anabolic effects achieved with antibody-mediated sclerostin inhibition.

Dr. Li received his medical degree from Harbin Medical University in 1986 and received his Ph.D. degree from the Hamamatsu University School of Medicine in 1998. He completed his post-doctoral training with Drs. Larry Raisz and Carol Pilbeam at University of Connecticut Health Center in 2001, where he investigated the function of prostaglandin receptor E2 in bone. Prior to joining Amgen in 2004, Dr. Li was at Isis Pharmaceuticals where he worked on the therapeutic potential of novel antisense agents.

New Targets for Osteoporosis

Richard Kremer, Bone and Mineral Unit, Department of Medicine, McGill University, Montreal, QC

Bone integrity depends largely on the balance between bone resorption and formation. The osteoclast has been the main target of osteoporosis therapies to counter osteoclastic bone resorption following estrogen deficiency of the menopause. In this population, anti-resorptive agents have been very effective in preventing bone fragility-related fractures. Bisphosphonates have been widely used to target the osteoclast with high potency and sustainaibility. They have the advantage of long retention in bone and therefore maintain their effectiveness long after their withdrawal. Recent evidence, however, indicate their potential harmful effect on bone with the emergence of atypical fractures in long term users of bisphosphonates. Another class of antiresorptive agents are specifically targeting the cytokine system responsible for bone turnover regulation, namely the RANK/RANKL system. Monoclonal antibodies against RANKL have proved very effective in reducing turnover and improving fracture outcome. In addition to post-menopausal osteoporosis they are also very effective in induced osteoporosis in patients treated with aromatase inhibitors in breast cancer as well as men treated for prostate cancer with LHRH analogs. Their advantage lies in their specificity and their quick reversibility upon withdrawal of administration. Although osteoclast

still remains the main target of therapy in the growing armamentarium of agents against osteoporosis, targeting the osteoblast to enhance bone formation has been the subject of intense research. To date, however, the only FDA approved anabolic therapy is teriparatide, a bone builder administered intermittently over a period of 20 months. Disadvantages include the relatively short duration of administration in this chronic disease, the need for daily self-administration by subcutaneous injection and the rapid disappearance of its effect following medication withdrawal. Hence, therapies specifically targeting the osteoblast through other signalling systems including the wnt signalling pathway are actively pursued. These include ant-DKK1 and ant-sclerostin monoclonal antibodies currently in phases 1 and 2 clinical trials. Finally, a different class of agents that seems to be beneficial through a combined inhibition of the osteoclast and stimulation of the osteoblast are also emerging. These include strontium ranelate, an agent approved in Europe but not in North America that seems to be regulating bone function through modulation of the calcium sensing receptor (CaSR) and cathepsin K inhibitors currently in phase 3 clinical trials.

Overall, the therapeutic strategies against osteoporosis are growing at a rapid pace and will without doubt lead to major improvements in this condition in the next few years.

Richard Kremer

Dr Richard Kremer is currently Professor and Director of the Bone and Mineral Unit in the Department of Medecine of McGill University. He is also the co-leader of the Musculoskeletal Axis of the McGill University Health Centre. Born in Paris, Dr Kremer received his MD and Ph.D. degrees at the Pierre et Marie Curie University of Paris, and completed his internal medicine and endocrinology residency at La Pitié-Salpêtrière Hospital in Paris. He then moved to Canada and completed his residency training at the Montreal General Hospital, Royal Victoria and Ottawa Civic Hospitals. He pursued his research training at the Royal Victoria Hospital from 1986-1989 and joined the Calcium Research Laboratory as a full-time physician-scientist. Dr Kremer is the recipient of several prizes and awards including the Chercheur National award from the Fonds de Recherches en Santé du Québec. He has made significant contributions to the Canadian Institutes of Health Research, the US National Institutes of Health and the US National Cancer Research Institute on grant and award committees. He is a reviewer for several international journals and is a consultant to industry, academic and lay organizations in his capacity as a clinician scientist and expert in metabolic bone diseases.

Thursday PM - Track 3/4

Chronic Diseases, Infection and Immunity

Inhibition of Quorum Sensing and Biofilm Formation by Tropical Plants (NHPRS-06-01)

Chieu Anh Ta[1], Marie Freundorfer[1], Ammar Saleem[1], Ana Gargaun[2], Mario Garcia Quesada[3], Marco Otorola Rojas[3], Pablo Sanchez-Vindas[3], Luis Poveda[3], Victor Cal[4], Tony Durst[2], and John T. Arnason[1].
[1]Centre for Research in Biopharma-ceutical and Biotechnology, Department of Biology, University of Ottawa, Ottawa, Ontario; [2]Department of Chemistry, University of Ottawa, Ottawa, Canada; [3]Herbario Juvenal Valerio Rodriguez, Escuela De Ciencias Ambientales, Universidad Nacional, Costa Rica; [4]Belize Indigenous Training Institute, Punta Gorda, Belize. Email: john.arnason@uottawa.ca

This is a new approach to the discovery of new phytochemicals from tropical plants that can interfere with the formation of bacterial biofilms. Bacteria use a cell-to-cell communication system known as quorum sensing (QS) to coordinate gene expression for the formations of these biofilms. Ethanolic extracts of tropical and traditional anti-infective plants were screened for QS interference and biofilm inhibition. Extracts from the Melastomataceae, Meliceae, Combretaceae, and Euphorbiaceae showed the highest inhibitory activities. In particular, one Melastomataceae species (Oxlaju' cha'jom) was most promising with QS inhibition zone of 27.7 + 0.6 mm and biofilm MIC (minimum inhibitory concentration) of 50 μg/mL. Interestingly, this is the first report of biological activity for this plant and very little is known about its phytochemistry. Bioassay-guided fraction of Oxlaju' cha'jom showed that inhibitory activities are in the more polar fractions. Current work is being done on the isolation and identification of the active principles.

Immunomodulatory and Anti-allergy Effects of CVT-E002, A Patented Proprietary Extract of North American Ginseng (NHPRS-06-02)

Cory Ebeling[1], John R. Gordon[2], Darryl J. Adamko1, Dilini Vethanayagam[2], Andrew Cave[3], Christopher Sikora[4], Ronald Filderman5, Karen Filderman[5], Allan Knight[6], Paul Keith[7], Gerald Predy[8], Katherine A. Rittenbach9, Christine Lutsiak[9], Sharla K. Sutherland[9], Jacqueline J. Shan[9].
[1]Pulmonary Research Group, Department of Medicine and Pediatrics, University of Alberta, Edmonton;. [2]Division of Pulmonary Medicine, Department of Medicine, University of Alberta, Edmonton; [3]Department of Family Medicine, University of Alberta, Edmonton; [4]Community Medicine Resident, University of Alberta, Edmonton; [5]Melimar Allergy Laboratory Inc. [6]JDM Research, Toronto, Canada [7]Department of Medicine, Division of Allergy and Clinical Immunology, McMaster University, Hamilton, Canada [8]Alberta Health Services, Edmonton; [9]Afexa Life Sciences Inc., Edmonton, Canada
Email: krittenbach@afexa.com

Allergic rhinitis is a disorder of the upper airways characterized by Type I hypersensitivity reactions, and is mediated by IgE antibodies produced in response to inhaled allergens. The aberrant IgE production is driven by an imbalance in the ratio of T helper 1 (Th1) to T helper 2 (Th2) immune responses. Current allergy treatments focus on symptom control rather than correction of the underlying immune issues. CVT-E002 (an active ingredient in COLD-FX®, IMMUNITY-FX®; isolated from *Panax quinquefolius*) is a unique, patented ChemBioPrint-developed and -standardized polysaccharide extract that has been shown to function as an immunomodulator that alters the intensity as well as the type of immune response. Previous studies have shown CVT-E002 may normalize the ratio of Th2:Th1, suggesting

exploration of its therapeutic potential in the context of allergy is warranted. In the experiments described here, CVT-E002 was studied in a mouse model of atopic asthma. Mice that had been previously sensitized to ovalbumin (OVA) were treated with either CVT-E002 or vehicle. Following treatment, mice were challenged intranasally with OVA and assessed for airway hyperresponsiveness and eosinophilic airway infiltration. CVT-E002 treatment significantly inhibited both the airway hyperresponsiveness and the amount of eosinophilic infiltration in the lung. These data suggest CVT-E002 may be effective in reducing allergy symptoms in healthy adults. To further test this hypothesis, a Canadian multi-centre, randomized, double-blind, placebo-controlled clinical trial was conducted to investigate the safety and efficacy of four weeks of CVT-E002 (200 mg bid) intake versus placebo in 200 healthy adults with confirmed seasonal allergic rhinitis.

Inhibition of Angiotensin Converting Enzyme (ACE) by Wild Berry Extracts in Vitro (NHPRS-06-03)

B.W. Nileeka Balasuriya and H.P. Vasantha Rurpasinghe.
Department of Environmental Sciences, Nova Scotia Agricultural College, Truro, Nova Scotia, Canada. Email:vrupasinghe@nsac.ca

Hypertension is an independent risk factor in development of cardiovascular diseases. As the global burden of hypertension is increasing it is vital to find measurements of prevention and cure. Natural health products are gaining recognition as a leading factor in prevention and treatment of most chronic diseases. In the current study five berry extracts were examined for their ability to inhibit angiotensin converting enzyme (ACE) which is a key enzyme in regulating blood pressure. Blueberry (*Vaccinium corymbosum*), cranberry (*V. macrocarpon*), partridgeberry (*V. vitis-idaea*), crowberry (*Empetrum nigrum*), and cloudberry (*Rubus chamaemorus* L.) were among the selected types of berries. Enzyme inhibition was determined using a fluorescence based assay at the presence of histidine-L-hippuryl-L-histidine-chloride substrate. Ethanol extracts of berries were investigated on their concentration dependant enzyme inhibition. All the berry extracts showed a concentration responsive enzyme inhibition in vitro indicating effective ACE inhibition. However, partridgeberry and cranberry were among the most effective ($p=0.05$). The study provided valuable insights on potential antihypertensive properties of bioactives of wild berries and further investigations are underway using cell lines and research animal models.

"Crawling in the Shadows": The Access Experience of Users of Cannabis for Therapeutic Purposes (NHPRS-06-04)

Lynda G. Balneaves[1], Rielle Capler[2], Bindy K. Kang[1], and Joan L. Bottorff[3].
[1]UBC School of Nursing, Vancouver, BC; [2]Canadians for Safe Access [3]UBC Okanagan, Kelowna, BC, Canada.
Email: lynda.balneaves@nursing.ubc.ca

With the growing evidence supporting the efficacy of cannabis for a range of medical conditions has been a corresponding increase in cannabis use for therapeutic purposes (CTP) in Canada. In British Columbia, 1/3 of cannabis users report using cannabis as a therapeutic agent. In recognition of the potential benefits of cannabis, the Medical Marihuana Access Division (MMAD) was developed by Health Canada to regulate access to cannabis for individuals with confirmed medical conditions. The purpose of this qualitative study was to understand the access experiences of CTP users. In-depth qualitative interviews were conducted with 23 CTP users. The majority of users accessed cannabis through community dispensaries (n=20), followed by licensed (n=10) or non-licensed growers (n=10), and Health Canada (n=5). CTP users' experiences in accessing cannabis mirrored the struggles they experienced in accessing the health care system. Many reported being "fearful" of requesting cannabis because of concerns that it would negatively impact their care and relationship with their physician, result in them being labelled as a "drug addict" or "criminal", or jeopardize their anonymity. As a result, CTP users engaged in an elaborate preparation process prior to requesting cannabis to overcome the systematic and social barriers they perceived to exist. While some CTP users were supported in their requests, others experienced negative reactions that required them to seek cannabis from non-legal sources. The findings suggest that revisions are needed to Health Canada's federal program as well as education for health professionals to address the barriers to CTP experienced by patients.

Thursday PM - Track 3

NHP Product Quality

Method Development Studies for the Determination of Aloin A and B in Aloe Vera Leaf Juice Ingredients and Products by High Performance Liquid Chromatography with UV Detection (NHPRS-07—01)

Paula N. Brown[1], Jamie Finley[1], Chiow Kuan[1], Steven Dentali[2], Ronan Yu[1], Paul R. Shipley[3].
[1]BC Institute of Technology, Burnaby, BC, Canada; [2]American Herbal Products Association, Silver Spring, MD, USA; [3]Department of Chemistry, University of British Columbia Okanagan, Kelowna, BC, Canada. Email: Paula_Brown@bcit.ca

The plant *Aloe vera*, the source of aloe vera leaf juice used in foods, cosmetics, and dietary supplements, has been historically recognized for its numerous beneficial uses including burn treatment and promotion of skin healing. The commercial production of aloe vera leaf juice is accomplished by removal of the leaf rind followed by isolation of the inner leaf gelatinous material, or by filtration and decolorization of whole leaf puree with activated charcoal. Both approaches minimize the amount of anthraquinone-rich aloe latex in the resultant aloe leaf juice product. Anthraquinones, a class of compounds suspected of having carcinogenic properties, are present as the glycosides, aloe-emodin, aloin A and aloin B in Aloe vera. The International Aloe Science Council has established a limit of 10 ppm for anthraquinone content in aloe vera leaf juice products intended for oral consumption. Extraction and chromatographic optimization studies were undertaken to support the development of an accurate, precise and efficient analytical method for determining aloin A and aloin B in aloe vera leaf juice products. This method was employed to determine aloin A and aloin B content in charcoal-treated (decolorized) aloe vera leaf juice, untreated (non decolorized) aloe vera leaf juice, and for comparison with an aloe vera leaf juice concentrate described as "aloe vera non-decolorized whole leaf extract" in a draft technical report, "Toxicology and Carcinogenesis Studies of a Nondecolorized Whole Leaf Extract of *Aloe Barbadensis* Miller (Aloe Vera) in F344/N Rats and B6C3F1 Mice (Drinking Water Study)" released by the US National Toxicology Program.

Creating GMP Specifications for Dietary Supplement Ingredients and Finished Products: What is Feasible? (NHPRS-07-02)

Kerri l. LeVanseler. NSF International, Ann Arbor, MI, USA. Email: levanseler@nsf.org

With GMP requirements fully in place for Dietary Supplements and Natural Health products in the United States and Canada, it is a time for action by the manufacturers. Specifications must be available for each ingredient and each finished product. Ingredient specifications need to cover identification, assay testing, contaminant limits and other critical aspects associated with each in-coming material. For finished product specifications, contaminant and assay testing remain essential but the test matrix can become much more complicated. The methods used must be valid and fit for purpose in the finished product sample. This presentation will discuss real scenarios and discuss what is feasible and where challenges exist. Examples will be provided to help to create specifications that focus on critical quality aspects with recognition of the resource restraints that exist. Problem situations and potential resolutions from a 3rd party certification perspective will be discussed. The utilization of "ANSI/NSF Standard 173 Dietary Supplements" as a guidance document for specification development will also be addressed.

Nuclear Magnetic Resonance Based Screening Tool for Quality Control of Botanicals (NHPRS-07-03)

Joshua M. Hicks[1], Christian Fischer[2], Sarah Luchsinger[1], Kristina McIntyre[3], Jonathan Ferrier[3], John T. Arnason[3], Alain Cuerrier[4], Eleni Yiantsidis[5], Catherine Neto[5], and Kimberly L. Colson[1].
[1]Bruker BioSpin, Billerica, MA, USA; [2]Bruker BioSpin GmbH, Rheinstetten, Germany; [3]Dept. of Biology, Univ. of Ottawa, Ontario, Canada; [4]Montreal Botanical Garden, Montreal, Canada; [5]Department of Chemistry and Biochemistry, University of Massachusetts-Dartmouth, North Dartmouth, MA
USA Email: kim.colson@bruker-biospin.com

Assessment of quality of botanical dietary supplements is challenging due to the complex nature of the molecular components that vary with growing location, seasonal conditions, harvesting conditions and processing conditions. The ability of Nuclear Magnetic Resonance Spectroscopy (NMR) to analyze complex mixtures as a non-targeted fingerprinting method combined with rapid sample preparation makes it an attractive analytical tool for the routine quality control analysis of botanical extracts. In this presentation we will show our work towards developing an NMR based quality control tool for crude botanical extracts such as ginseng, cranberry and blueberry. This presentation includes (1) evaluation of NMR reproducibility in a multisite validation study to establish robust screening methods, (2) statistical methods used to characterize the botanical extracts, (3) sample characterization to provide information such as the varietal, sample purity, and natural variation in samples, and (4) identification of the presence of single components within a crude extract and quantification of these components.

NMR-based Metabolomic Profiling of *Vaccinium macrocarpon* Aiton (NHPRS-07-04)

Paula N. Brown, Ryan S. Lohre, Paul R. Shipley.
Department of Chemistry, University of British Columbia Okanagan, Kelowna, British Columbia, Canada. Email: paul.shipley@ubc.ca

Cranberries (*Vaccinium macrocarpon* Aiton) and cranberry juice have been used since the early 19th century for treatment of UTIs and over the past decade a large body of clinical evidence has emerged that supports this use. The mechanism of action of cranberry has not been clearly determined and studies have focused primarily on a limited number of specific classes of compounds. Recent publications indicate that NMR-based metabolomics is an effective tool for understanding the chemical diversity and complexity in natural products. There is no currently published data describing the entire cranberry metabolome or the diversity between individual cranberry fruit. To explore the chemodiversity in the cranberry fruit metabolome as a function of cranberry harvest, nuclear magnetic resonance spectrometry-based metabolomic profiling of cranberry fruit harvested from 22 farms across the Vancouver Lower Mainland of British Columbia was undertaken. Optimized protocols for acquiring metabolomic profiles of cranberry fruit by NMR spectrometry were developed and different approaches to multivariate data treatment explored.

Thursday PM - Track 4

NHP Drug Interactions

Inactivation of Active Vitamin D3 is Inhibited by Drug Interactions in Human liver (NHPRS-08-01)

Subrata Deb, Hans Adomat, Emma Tomlinson Guns.
The Vancouver Prostate Centre, Vancouver, BC, Canada.

Lower serum levels of vitamin D_3 are associated with increased risk of prostate cancer, colon cancer and breast cancer. Similarly, acquired immunodeficiency syndrome (AIDS) patients have lower vitamin D_3 levels, which may render them to further lowering of immune function, bone diseases and increased risk of opportunistic infections. Inactivation of $1\alpha,25\text{-}(OH)_2D_3$, the biologically active form of vitamin D_3, by cytochrome P450 3A4 (CYP3A4) enzyme can be an important determinant of it's serum and tissue levels. The purpose of the present study was to investigate the metabolism of $1\alpha,25\text{-}(OH)_2D_3$ in human liver microsomes and recombinant CYP3A4 to it's inactive hydroxy metabolites and to evaluate the inhibitory potential of various medications commonly used by cancer (e.g. ketoconazole, tamoxifen, taxanes) and AIDS (e.g. ritonavir, clarithromycin) patients. A liquid chromatography-mass spectrometry method was developed to analyze metabolites of $1\alpha,25\text{-}(OH)_2D_3$. The metabolite formation pattern was similar in human liver microsomes and human recombinant CYP3A4 with the emergence of multiple metabolites following incubation with $1\alpha,25\text{-}(OH)_2D_3$. Co-incubation of $1\alpha,25\text{-}(OH)_2D_3$ with ketoconazole, tamoxifen, docetaxel, paclitaxel, ritonavir or clarithromycin at varying concentrations led to approximately 60-100% inhibition of $1\alpha,25\text{-}(OH)_2D_3$ inactivation. Similar inhibitory effect was also observed on triazolam hydroxylation (1`-OH and 4-OH), a human CYP3A4 marker assay, following co-incubation with these drugs. IC50 values were determined using GraphPad Prism nonlinear regression analysis. In summary, our results suggest that medications commonly used to treat cancer and AIDS patients may be beneficial in maintaining the optimum levels of $1\alpha,25\text{-}(OH)_2D_3$ by inhibiting CYP3A4-mediated inactivation of active vitamin D_3 in human liver.

In vitro Effects of Cree Anti-Diabetic Herbal Medicines Against: The Metabolism of Repaglinide (NHPRS-08-02)

Rui Liu[1], Anthony Krantis[1], Ammar Salem[1], J. Thor Arnason[1], Brian C. Foster[1,2].
[1]Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, Ottawa, ON; [2]Therapeutic Products Directorate, Health Canada, Ottawa, ON, Canada

Background: The Cree Nation of Eeyou Istchee (CEI) of Northern Quebec has a high prevalence of Type II diabetes and uses a number of herbal medicines to treat the symptoms of diabetes. However, their metabolism profiles and capability to interact with conventional therapeutic products are not known. As these Cree medicines will be used as both alternative and complementary medicines it is critical to determine their capacity to affect drug metabolism, potentially affecting the efficacy and safety of drugs or other herbal medicines.
Purpose: This study was performed to characterize the effect of selected Cree anti-diabetic herbal medicinal products on the metabolism of repaglinide, one of the widely used meglitinide class of blood glucose-lowering drugs.
Methods: Ethanol extracts from of 17 plant species (AD01-AD03, AD06-AD09, AD11, W1-W9) were prepared in methanol (10 mg/ml). Extracts were examined for their effects on the formation of major repaglinide metabolites by using established in vitro bioassays with human liver microsomes.
Results: Our data indicates that most of 17 Cree anti Cree anti-diabetic herbal extracts showed strong

inhibitory effect on the metabolism of repaglinide except W9. The top 5 most inhibitory products are AD01, AD06, AD11, W1 and W2 which inhibited 90% of the repaglinide metabolism.
Conclusions: According to our study, most of the 17 Cree anti Cree anti-diabetic herbal extracts exhibit the potential to interact with repaglinide. They may inhibit the metabolism of repaglinide and cause overdose. Further studies are warranted to determine if these effects are clinically significant.

Clinical Development of Herbal Medicines: Cuban Regulatory Perspectives (NHPRS-08-03)

Diadelis Remirez, Deybis Orta.
National Center for State Quality Control of Drug.

Cuba has a prodigious flora that offers therapeutic alternatives to Public Health and Veterinary Medicine. New investigations are being carried out in order to get natural health product (NHP). The traditional medicine has played an important role in the treatment of diverse pathologies, mainly in the developing countries. This work shows the importance of a rigorous testing of herbal medicine as alternative therapeutic for human use. Besides that, the policies of clinical trials as well as the value of Good Clinical Practice in order to guarantee the safety, quality and efficacy of NHP are shown; the main mistakes in Clinical Trials of natural products are also explained. The strategies for the development of herbal medicinal products in Cuba are showed. The natural health products are considered a very important source for the health in Cuba. The market and the main challenges are analysed in the investigation of the phytomedicines.

Ethnopharmacological Investigation of Medicinal Plants from Peru

Gabriel Picard[1], John Arnason[1], Rosario Rojas[2], Carolos Bueno[2], Joaquina Alban Castillo[3].
University of Ottawa[1], Universidad Peruana Cayetano Heredia, Universidad Nacional Mayor de San Marcos[3].

The neotropical Piperaceae have been shown to have significant neuropharmacological properties from our previous research program. In the current work, we collected a total of 54 Piperaceae Peruvian Piperaceae plants for study from April to July of 2009. Plants used traditionally to treat epilepsy, anxiety related disorders, and culture bound syndromes such as susto and mal aire were targeted. In pharmacological tests for activity in targets relevant to these uses, the $GABA_A$-BZD Receptor binding assay and Gaba transaminase inhibition, a large number of active species were found. Bioassay guided isolation of active compounds from selected species led to identification of chromenes, lignans and piperamidesas the active principles. The results suggest that the traditional use of these plants has a pharmacological basis.

Health Product Interactions (NHPRS-08-04)

Brian C. Foster[1,2].
[1]Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, Ottawa, ON; [2]Therapeutic Products Directorate, Health Canada, Ottawa, ON, Canada.

Substances within all health products (biologics, drugs, foods and natural health products) have the potential to interact with other health products. The interaction may be either pharmacokinetic or pharmacodynamic in nature resulting in decreased, increased (boosted, pharmacoenhanced) or unchanged activity. The reversible or irreversible interactions occur when substances within health products compete for the same receptor, metabolic enzyme or transport protein active sites or shunt the substance and their metabolites through other pathways. The interaction may be associated with the pharmacologically active agent or another substance such as an excipient or botanical constituent such as the furanocumarins present in grapefruit juice and other foods. Health products can affect more than enzyme and protein such that clinically relevant interactions adversely affecting the safety and efficacy of health products are possible, particularly in populations on multiple products or with polymorphisms affecting disposition. The presentation will examine the potential of health products to affect the metabolism of human cytochrome P450 isoforms and transport, in vitro models for interaction studies, and how in vitro results may translate to the clinical situation.

Friday 9:30 AM - Track 1

Dr. Richard Ogilvie Lecture

Drugs and People I have Known: 45 Years in Clinical Pharmacology.

Richard I. Ogilvie, Professor Emeritus of Medicine and Pharmacology, University of Toronto; Clinical Pharmacologist, Hypertension Unit, Toronto Western Hospital

This lecture provides an opportunity to review my interactions with teachers, mentors, fellows and associates over 45 years in Clinical Pharmacology. My teachers and mentors include several who have resonated strongly in pharmacology including Lucas, Kalow, Best, Ruedy, Melville, Nickerson and Beaulnes. I rarely think of any drug , trial, investigation or concept, without associating a name with the drug: thalidomide (Kalow, Kelsey), mersalyl (Melville), acetazolamide (Zborowska, Klassen), ethacrynic acid /furosemide (Ruedy, Perez), chlorthalidone (Tweeddale), digoxin (Klassen, Ruedy), theophylline (Mitenko, Piafsky), tolbutamide (Zinman, Loubatières), methyldopa (Oates), timolol (Dorian, Achong), diazoxide (Sitar, Nadeau), amantadine (Aoki, Sitar), adverse drug reactions (Ruedy), non-medical use of drugs (Beaulnes), teaching pharmacology (Ruedy), prescribing models (Kreeft), plateau concentrations (Mitenko), hypotension, drug withdrawal (Rangno), forearm circulation/metabolism (Klassen), and models of the circulation (Larochelle). Even organizations that promoted clinical pharmacology in Canada have names embedded in my memory: CFAT (Murphy, Nash), ASPET, IUPHAR (Melville, Sjoqvist), Oenophile Society (Ruedy, MacLeod), and CSCP (Sellers, MacLeod, Mahon). Over 50 residents and fellows have assisted me in research and teaching. My career as an individual rather than a population clinical pharmacologist has been populated with many people! Interactions between people are even more interesting than interactions between drugs. It is my fondest hope for many more, *Tout Jour à Fin!*

Richard Ian Ogilvie
Dr. Ogilvie was born in Sudbury and completed his medical degree at the University of Toronto in 1960. After interning at the Toronto General Hospital and a year in general practice in Copper Cliff, Ontario, he trained in Internal Medicine at the Montreal General Hospital, followed by Clinical Pharmacology under the mentorship of Dr. John Ruedy. He joined the Faculty of Medicine at McGill, became Director of Clinical Pharmacology, and by 1978, Professor and Chair of Pharmacology and Therapeutics. In 1983 he moved to the Toronto Western Hospital as Chief of Cardiology and Clinical Pharmacology. His research publications and teaching efforts have been in the area of cardiovascular clinical pharmacology with a special interest in hypertension. He was the Editor of *Hypertension Canada* from 1989 to 2008. He was the founding President of the Canadian Society for Clinical Pharmacology and has served as a Director and President of the Canadian Hypertension Society and Chair of IUPHAR Clinical Pharmacology Section. He continues to work in the Hypertension Unit of the Toronto Western Hospital and is a member of the Diabetes Recommendations group of CHEP.

Friday 9:30 AM - Track 2

Drug Delivery

Medical Nanorobotics for Enhanced Drug Delivery Through the Human Vascular Network – Cancer Application

Sylvain Martel, Professor & Director, NanoRobotics Laboratory, Ecole Polytechnique Montreal, QC

In recent years, medical robotics have evolved from interventions being performed by large robots outside the patient to smaller untethered versions such as the camera pills capable of travelling through the digestive track. More recently, nanotechnology and robotics were combined to develop new interventional platforms designed to navigate therapeutic carriers capable of targeting regions in the human body such as tumors only accessible through smaller diameter blood vessels. These new navigable therapeutic agents could play a major role in cancer therapy by enhancing therapeutic efficacy by delivering an improved concentration of the drug at the targeted area while decreasing systemic side effects compared to modern interventions such as chemotherapy. The talk will begin with one example of such navigable agents that was successfully synthesized and which has been referred to as Therapeutic Magnetic Micro-Carriers (TMMC). TMMC are biodegradable polymer encapsulating magnetic nanoparticles and a therapeutic load. The nanoparticles are used not only to navigate the carriers in the blood vessels using an upgraded clinical Magnetic Resonance Imaging (MRI) scanner but allowed them to be visible using the same scanner. Recent *in vivo* experiments validated the potential of TMMC in interventions such as liver chemoembolization where the cancer drugs carried by the TMMC were successfully released after targeting the left or right liver lobes of rabbits. Then another type of navigable agents designed to deliver therapeutic loads to colorectal tumors will be presented. They consist of drug-loaded MC-1 magnetotactic bacteria that can be controlled by an external computer and being visible deep in the body using MRI. Then, examples with videos will show the possibility of integrating these two complementary agents in order to increase the range of cancers that could be treated by such new approach based on targeted delivery of navigable therapeutics.

Sylvain Martel
Sylvain Martel received the Ph.D. degree in Electrical Engineering from McGill University, Institute of Biomedical Engineering, Montréal, Canada, in 1997. Following postdoctoral studies at the Massachusetts Institute of Technology (MIT), he was appointed Research Scientist at the BioInstrumentation Laboratory, Department of Mechanical Engineering at MIT. From Feb. 2001 to Sept. 2004, he had dual appointments at MIT and as Assistant Professor in the Department of Electrical and Computer Engineering, and the Institute of Biomedical Engineering at École Polytechnique de Montréal (EPM), Campus of the University of Montréal, Montréal, Canada. He is currently Professor in the Department of Computer and Software Engineering, and the Institute of Biomedical Engineering, and Director of the NanoRobotics Laboratory at EPM that he founded in 2002. Dr. Martel holds the Canada Research Chair (CRC) in Micro/Nanosystem Development, Fabrication and Validation since 2001 and he is a Fellow of the Canadian Academy of Engineering. In the medical alone, he pioneered several innovative technologies such as the first parallel computing platform for remote surgeries, direct cardiac mapping systems designed to investigate the cause of sudden cardiac deaths, and new brain implants for decoding neuronal activities in the motor cortex. Presently, Dr. Martel is leading an interdisciplinary team involved in the development of new types of therapeutic agents and interventional platforms for cancer therapy.

Friday 8:30 AM - Track 3/4

Traditional Western Herbalism: Opportunities and Challenges in Collaborative Research

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The Clinical Herbalist: A Key to Unlocking Good Herbal Research

David Winston, Herbalist and Ethnobotanist

As herbal products have become more popular, clinical research into the uses and effects of these products has grown as well. In most clinical research, teams of physicians, phytochemists, pharmacists, pharmacognocists and statisticians, design and implement these studies. While much good research comes from such groups, a significant number of poorly thought-out studies, failed studies and inaccurate studies comes from the fact that the people who are most familiar with the medicinal use of herbs, play no role. The clinical herbalist is familiar with traditional preparations (fresh vs. dried plant material, water vs. alcohol soluble constituents), the parts used (i.e. kava root, rather than the potentially hepatotoxic stem peelings), historical/traditional uses of herbs, how they are combined to enhance absorption or reduce toxicity and adequate dosage levels needed for clinical efficacy. I this talk I will present examples of flawed research and explain how a clinical herbalist as part of a design team can help make medicinal plant research more productive.

David Winston
David Winston is an Herbalist and Ethnobotanist with over 40 years of training in Cherokee, Chinese and Western herbal traditions. He has been in clinical practice for over 34 years and is an herbal consultant to physicians, herbalists and researchers throughout the USA and Canada.

David is the founder/director of the Herbal Therapeutics Research Library and the dean of David Winston's Center for Herbal Studies, a two-year training program in clinical herbal medicine. He is an internationally known lecturer and teaches frequently at medical schools, symposia and herb conferences. He is also the president of Herbalist & Alchemist, Inc. an herbal manufacturing company,

David is a contributing author of *American Herbalism,* published in 1992 by Crossings Press, the author of *Saw Palmetto for Men & Women*, Storey, 1999 and *Herbal Therapeutics, Specific Indications For Herbs & Herbal Formulas*, HTRL, 2009 (9th edition) and the co-author of Adaptogen's Herbs for Strength, Stamina and Stress Relief, Healing Arts Press, 2007, and Winston and Kuhn's *Herbal Therapy and Supplements; A Scientific and Traditional Approach,* Wolters Kluwer/Lippincott, 2008. In addition, David is a founding/professional member of the American Herbalist Guild, and he has served four terms on the Board of Directors.

The Value of Herbalists in Collaborative Research

Daniel Gagnon, Herbs, Etc., Santa Fe, New Mexico, USA

When medical herbalists consult phytotherapy books to learn about an herb, they often use the reference section to locate papers or studies about the herb. They may also search the web and find papers related to their subject of interest. What they are often unaware of is that the vast majority of these studies have not been done or devised by or with the help of herbalists. In most cases, an herbalist was never consulted and no concerns about the possible clinical application of the results were anticipated. In other cases, herbal research has been conducted by phytopharmaceutical companies. The researchers' concerns are usually focused on specific outcomes related to the manufacturers' products. In some countries, the governmental regulatory body

overseeing phytotherapy manufacturers may require them to do such research in order to gain marketing rights. It is apparent that phytotherapy research is often disconnected from clinical applications. An exceedingly small percentage of the research has end points that are applicable to practicing medical herbalists. Herbalists must extract practical information and possible therapeutic applications from each and every paper. With these concerns in mind, this presentation explores ways to bridge the gap between academia and practicing herbalist with the goal to share results and findings relevant to clinical practice.

Daniel Gagnon

Daniel Gagnon, owner of Herbs, Etc., resides in Santa Fe, New Mexico, and has been a practicing Medical Herbalist since 1976. Born in French Canada, he relocated to Santa Fe in 1979. There, he furthered his studies at the Santa Fe College of Natural Medicine and the College of Pharmacy at the University of New Mexico. In 2004, he obtained his Bachelor of Science in Herbal Medicine from the North American College of Botanical Medicine in Albuquerque. He has submitted his thesis on laboratory assays of the antimicrobial activity of hops (Humulus lupulus) on methicillin-resistant Staphylococcus aureus (MRSA) for his Masters Degree at the Scottish School of Herbal Medicine. Daniel is the author of the best-selling book Liquid Herbal Drops in Everyday Use (over 120,000 books sold to date) and co-author of Breathe Free, a nutritional and herbal self-care book for the respiratory system.

Taking Research a Step Forward, Teaming With a Traditional Herbalist

Marie Provost, Traditional Herbalist, Val-David, QC

For centuries, herbalists built their expertise and their successes with the sole tools of intuition, experience and communication with other herbalists. Empericism was useful in separating good from bad remedies as mothers, healers and elders would pass on their teachings.

Within the last decade, herbalism has evolved through the next level: being recognised and promoted (for example, the WHO has stressed the importance of traditional medicines in 2009). Recently, scientific research has been more receptive to herbalist's concerns and to medicinal plants, which triggered increased data regarding dosage and contraindications.

We are now at the edge of yet another shift: knowledge expansion and fusion of herbalism with the general medical practice. Western herbalists do not seek to value their practice as much as to better understand how to use plants in a more efficient and safe way.

Science and its methods offer a unique tool to herbalists who are looking at evaluating medicinal plant on their patient health. Such tools are numerous and lead the way to: 1) a better understanding of patient health, 2) more efficient extraction methods which respect the whole plant (crude extracts), 3) an increased awareness of possible drug-plant interactions.

Scientific researchers must work in collaboration with Western herbalists and need to take cognisance of what is happening within the lab and expertise of herbalists.

Marie Provost

Marie Provost has always been interested in medicinal plants and their properties. Visionary herbalist, a pioneer of traditional herbalism in Quebec, she founded l'Herboristerie la Clef des Champs in 1978 and is its Director General to this day.

Founding member of Guilde des herboristes (today 350 members), she was president of its Executive Council for 11 years until 2006. A defendor of free access to medicinal plants, she represented traditional herbalism at the Natural Health Product Directorate and sat on the Management Advisory Committee (Health Canada) for 4 years. She is also a founding member of Table Filière des Plantes Médicinales Biologiques, has been on its Executive Council since 1999 and has been its President since 2007.

A firm believer that the future lies in the collaboration between the scientific and traditional communities, she has worked diligently to establish bridges and promote a dialogue between regulatory authorities, scientific researchers and traditional herbalists.

Friday 10:45 AM - Track 1

Hypertension

Reactive Oxygen Species, Redox Signalling and Vascular Biology

Rhian M Touyz, University of Ottawa

Molecular mechanisms contributing to the pathoetiology of vascular disease, such as hypertension, are complex, involving many interacting systems such as signaling through G protein-coupled receptors, the renin-angiotensin system, vascular inflammation and remodeling, vascular senescence and aging and developmental programming. Common to these systems is NADPH oxidase-derived reactive oxygen species (ROS). ROS play a major role as intracellular signaling molecules in the regulation of vascular function and structure. In pathological conditions, loss of redox homeostasis contributes to vascular oxidative damage. Specific enzymes, the Nox family of NADPH oxidases, have the sole function of generating ROS in a highly regulated fashion in physiological conditions, and that in disease states, hyperactivation of Noxes contributes to oxidative stress and consequent cardiovascular and renal injury. The Nox family comprises seven members, Nox1-Nox7. Nox1, Nox2 (gp91phox-containing NADPH oxidase), Nox4 and Nox5 have been identified in the cardiovascular-renal systems and have been implicated in cardiovascular and renal disease. Noxes, which are differentially regulated in hypertension, are major sources of cardiovascular and renal oxidative stress. This has evoked considerable interest because of the possibilities that therapies targeted against specific Nox isoforms to decrease ROS generation or to increase nitric oxide availability or both may be useful in minimizing vascular injury and renal dysfunction, and thereby prevent or regress target organ damage associated with hypertension. Despite convincing experimental evidence indicating a role for Nox-derived ROS in the pathophysiology of hypertension, clinical evidence remains controversial. This presentation provides current insights on Nox-mediated ROS generation and vascular effects of oxidative stress and discuss the significance of oxidative damage in experimental and clinical hypertension.

Rhian Touyz, MBBCh, MSc(Med), PhD, is the Canada Research Chair in Hypertension at the Kidney Research Centre, Ottawa Hospital Research Institute (OHRI)/Univ of Ottawa. She is a Senior Scientist at the OHRI and professor of medicine in the Division of Nephrology. Dr Touyz received her BSc(Hons) (1980), MBBCh (1984), MSc (1986) and PhD (1992) from the University of the Witwatersrand, Johannesburg, South Africa. She completed a post-doctoral fellowship (1992-1996) at the Clinical Research Institute of Montreal.

She has received numerous academic and research awards, including the Young Investigator Awards from the American Society of Hypertension, the Canadian Society of Hypertension and the Quebec Society of Hypertension as well as being a finalist for the Irving H Page Award, Atherosclerosis Thrombosis and Vascular Biology from the American Heart Association. Dr Touyz was awarded the 2005 Dahl Lecture Award by the American Heart Association, the 2006 Grace A Goldsmith Award, American Society of Nutrition, the 2009 Vincenzo Panagia Distinguished Lecture Award, Institute of Cardiovascular Sciences Award and the 2010 Distinguished Service award from Hypertension Canada, in recognition of her contributions to hypertension research. Dr Touyz co-chairs the Recommendations Task Force of the Canadian Hypertension Education Program (CHEP), she is the secretariat of the International Society of Hypertension and she is the past President of the Canadian Hypertension Society. She is the current Chair of the Council for High Blood Pressure Research, AHA. She is the Scientific Officer of the Cardiovascular committees at the Canadian Institutes of Health Research (CIHR) and the Heart and Stroke Foundation of Canada (HSFC) and she is a member of the Microcirculation and Hypertension

study section of the NIH. Dr Touyz chairs the organization committee for establishment of the Vascular Medicine and Biology program, Dept of Medicine, Univ of Ottawa. She is Associate Editor of Clinical Science and the Journal of the American Society of Hypertension and she is on the on the editorial board of numerous specialty journals, including Hypertension and Circulation Research.
Dr Touyz is funded by the CIHR, HSFC and JDRF. She has published over 250 original papers and more than 30 review articles. Her main focus of research relates to molecular, cellular and vascular mechanisms of hypertension. Her areas of study include clinical and experimental hypertension, hypertension in renal disease, signal transduction, oxidative stress, ion transport, vascular biology, adipose biology and insulin resistance and diabetes. She has a particular interest in translational research.

Practical Genomics in Cardiovascular Disease

Robert A. Hegele, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada

Evaluating the family history of illness has been a cornerstone of clinical assessment. We now may be close to replacing the family history with laboratory evaluation of a patient's genetic profile and disease risk, which could allow for more specific diagnosis, prognostication and rational selection of therapies. But while such tangible outcomes are being promoted by genomic scientists and geneticists, how realistic are these promises? Among the ~3 billion DNA bases in a single human genome, ~1% vary between any 2 individuals. Human genomic variation ranges from single base pair changes to alterations involving large segments of chromosomes. New technologies such as DNA microarray 'chips' and high-throughput DNA sequencing now enable low-cost, comprehensive examination of genomic variation, which can then be correlated with traits, such as blood pressure and hypertension. For instance, a popular research approach called the genome-wide association study (GWAS) evaluates patterns of association between single nucleotide polymorphism (SNP) genotypes and clinical phenotypes. Another approach uses high-throughout DNA sequencing to determine the impact of rare DNA mutations on clinical traits both in families and increasingly in populations. Currently, while proven genetic effects on clinical traits such as hypertension are statistically detectable in populations, they are usually modest. In contrast, some very rare and severe forms of hypertension result from mutation in a single gene: understanding pathogenesis in these families may be very important. Some commercial interests are beginning to market genomic DNA tests directly to consumers, so if only for this reason, it is important for clinicians to understand basic theory, methodology and interpretation of genomic analysis. In addition, selected DNA diagnostic methods and especially pharmacogenetics – differences in drug response due to genetic factors – may be poised to enter the clinic someday soon.

Robert Alexander Hegele
Jacob J. Wolfe Distinguished Medical Research Chair; Edith Schulich Vinet Canada Research Chair in Human Genetics, Tier I; Martha G. Blackburn Chair in Cardiovascular Research; Director London Regional Genomics Centre; Scientist, Vascular Biology Research Group, Robarts Research Institute; Distinguished University Professor, Departments of Medicine (Division of Endocrinology) and Biochemistry, University of Western Ontario.

Dr. Rob Hegele is an endocrinologist who runs the tertiary referral lipid clinic for Southwestern Ontario. He has treated over 4000 patients with cholesterol problems in his career.

His research in cholesterol and genetics has been funded continuously by the CIHR and the Heart and Stroke Foundation of Ontario since 1990. His lab's work was recognized with awards from the American Heart Association (AHA), the Canadian Diabetes Association, the American Society for Clinical Investigation, the Genetics Society of Canada and the Heart and Stroke Foundation of Canada, among many other national and international groups.

He has published >500 papers and has made >120 presentations at international meetings. He is among the top 1% of highly cited clinician scientists in the world. He has trained countless young physicians and graduate students in heart disease.

The Rapid Vascular Effects of Steroids: Sorting Out the Receptors and the Hormones

Robert Gros, Robarts Research Institute, Departments of Physiology & Pharmacology and Medicine, Schulich School of Medicine and Dentistry, University of Western Ontario, Canada

Vasoactive steroids are important physiological and pathophysiological regulators of cardiovascular function. The canonical mechanisms for vasoactive steroids, like aldosterone and estrogen, have focused on regulation of transcription via activation of their "traditional" cytosolic/nuclear mineralocorticoid (MR) and estrogen (ER) receptors. However scientists have speculated that these and other vasoactive steroids might have transcriptionally-independent effects mediating their rapid changes in smooth muscle contractile reactivity and regulation of cell growth/death, based on confounding findings going back more than half a century.

The mechanism(s) mediating rapid effects of steroids has remained a focus of controversy. Though the rapid effects of aldosterone and estrogen have been attributed to classical MR, ERα and ERβ receptors in some studies, but additional mechanism of actions for these responses have also been suggested. Recent studies have implicated GPR-30 (also called GPER) as a mediator of the rapid effects of estrogens. GPR-30, first characterized as an "orphan GPCR", is expressed on the cell surface and mediates its effects via several members of the G protein family of GTP-binding proteins. This receptor has been shown to contribute to the rapid effects of estrogen in several model systems. GPR30 is widely distributed and has been identified in both endothelial cells and vascular smooth muscle cells. To date, data have suggested that GPR30 is selectively activated by estradiol. However, our recent findings are consistent with the interpretation that estrogens are not the sole hormones which mediate their rapid effects via GPR30-dependent pathways and that aldosterone can mediate its rapid vascular effects potently via both GPR30- and MR-dependent mechanisms.

Robert Gros

Dr. Robert Gros completed his graduate training at the University of Western Ontario and fellowship training at the Toronto General Hospital. He is currently a scientist in the Vascular Biology Research Group at the Robarts Research Institute and an assistant professor in the Departments of Physiology & Pharmacology and Medicine at the University of Western Ontario. The focus of his research is to investigate the cellular/molecular mechanisms involved in the regulation of vascular function. With a particular interest in the role and regulation of G-protein-coupled receptor signaling pathways in both vascular smooth muscle and endothelial cell function under physiological and pathological conditions such as hypertension. He has been the recipient of the Evelyn McGloin Fellowship Award and the Dr. Maureen Andrew New Investigator Award from Heart and Stroke Foundation of Ontario. He was awarded the Young Investigator Award from the Canadian Hypertension Society and he currently holds a New Investigator Salary Award from the Heart and Stroke Foundation of Canada

Friday 10:45 AM - Track 2

Where the Breakthroughs Come From: Contribution of Biotech Companies

The State of Big Pharma

Elizabeth B. Vadas, President, InSciTech Inc., Dorval, QC

The last few years have seen unprecedented changes in the pharmaceutical industry, particularly in the world of large, multinational pharmaceutical companies. Many of these companies are faced with patent expirations of their major products along with pipelines that cannot be described as "healthy". Mergers and acquisitions have always been occurring in the industry, but in the last few years the pace has accelerated; in 2009 alone we have seen deals involving Merck and Schering-Plough, Pfizer and Wyeth, Roche and Genentech. Unfortunately M&A is a short term quick fix, but cannot be viewed as a long term solution to empty pipelines. It appears that the internal upheaval created by the merger does not fix a broken system and the purported efficiencies and synergies seldom materialize. In addition, large pharmaceutical companies have embraced the "contract everything out" philosophy to the point that some companies are divesting of their core competencies. The industry is under pressure from society also to create effective and safe new means of treating diseases at a lower cost.
In light of the reduced productivity of internal research engines, big pharma is looking for licensing opportunities to bring in new therapeutic agents to revitalize its business. Small drug discovery companies, be it small synthetic molecules or biologics, have a lot to offer to big pharma: by being innovative, daring to take intelligent risks, they create exciting new molecules based on sound science to fill the gaps in big pharma's pipelines.

Elizabeth B. Vadas
Elizabeth B. Vadas received her Ph.D. in Physical Chemistry from McGill University in Montreal. She obtained her undergraduate degree in colloid and surface chemistry in Budapest, Hungary. She joined Merck Frosst, the Canadian subsidiary of Merck & Co. in 1980 as a senior research scientist in the department of Pharmaceutical Research and Development. Over the years she has been involved in the formulation development of many new chemical entities discovered at the Merck Frosst Centre for Therapeutic Research while taking on increasing management responsibilities.

From 1991 to 2002 Dr. Vadas was head of Pharmaceutical Research and Development at Merck Frosst The department, under her leadership, was responsible from early compound characterization to formulation and process development of new chemical entities including the supply of clinical trial materials from phase I through phase III and technical transfer to manufacturing. Notable accomplishments of her department were several products developed for worldwide introduction. One of the most important of these products from the leukotriene program, leading to worldwide regulatory approval, was SINGULAIR®, Merck's once a day oral asthma therapy

In mid-2002 Dr. Vadas decided to take early retirement to establish her own consulting company, InSciTech Inc. Currently she works with several drug discovery companies both in the US and Canada providing development expertise.

Dr. Vadas has lectured and published widely. She is a Fellow of AAPS, Past President of the Canadian Society for Pharmaceutical Sciences and has been an adjunct professor of pharmaceutics at the University of Montreal for several years. She is the recipient of a number of scientific and management awards.

Pharmaceutical Biotech in the Prairies: The Genesis of Edmonton's Osteo-Metabolix Pharmaceuticals

Michael R. Doschak, Assistant Professor, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB

Osteo-Metabolix Pharmaceuticals Inc. (OMX) is a university spin-off company focused on the development, formulation and evaluation of bone-targeting biologics for bone conditions such as Osteoporosis, Paget's disease, and Osteoarthritis. OMX is well positioned to enter this market with a new exciting class of compounds based on bone seeking variants of the peptide hormone Calcitonin, which utilize covalently attached bisphosphonate moieties to provide significantly higher affinity for bone (note that the BP moieties are proven not to be involved in the therapeutic action, and serve to guide delivery of CT preferentially to bone).

OMX's patent-protected lead product, bisphosphonate conjugated Calcitonin (BP-CT), has been shown to significantly outperform current commercial formulations of Calcitonin in a preclinical rat model of Osteoporosis, whilst further reducing the major side effects associated with current monotherapy of either bisphosphonate or Calcitonin drugs alone. Accordingly, OMX are seeking a strategic co-development partnership for Lead Optimization of BP-CT to First Time in Human (FTIH) evaluation of their unique bone targeted therapy for bone disease.

OMX has further developed ``state of the art`` diagnostic capabilities for measuring bone turnover, that are required to validate its novel therapies. OMX is offering those unique advanced bone mass/mineral density imaging and bone toxicology assays on a fee-for-service basis, and they include:

1. High Resolution Micro-Computed Tomography Imaging of Bone: the non-invasive quantification of bone and mineral adaptations after pharmaceutical, orthopaedic, orthodontic and biomedical intervention;
2. *In vitro* Bone Toxicology / High-Throughput Cell Culture Assays. Advanced osteoclast and osteoblast culture systems for bone cell assay under GLP conditions to test novel drug or environmental chemical compounds for effects on bone cell stimulation, inhibition, and/or toxicity.

For further information, please visit us at: www.osteometabolix.com

Michael R. Doschak

Over the past 5 years, Mike Doschak has developed a strategic research program centered on characterizing the biological effects of drugs and peptide biologics with the mineralized connective tissues of the body, namely the bones and teeth. That research program in "Bone Health after Drug Intervention" followed from his Master's and Doctoral studies in the Medical Sciences at the University of Calgary, which employed non-invasive imaging technology, biomechanics and cell biology to detail the cellular and molecular actions of the bisphosphonate class of antiresorptive drugs, particularly following non-traditional therapeutic and/or diagnostic application in preclinical animal models of bone disease.

Following post-doctoral training in drug delivery and an NSERC Industrial Fellowship with the Canadian biotech company Millenium Biologix in Mississauga Ontario, he was recruited to the University of Alberta where he established the first *in vivo* Micro-Computed Tomography imaging lab in Edmonton, to temporally assess novel bone drug compounds on the mineralized tissues of small laboratory animals, non-invasively and at very high resolution. His research efforts recently culminated with the synthesis and characterization of several novel biologic drug compounds which many consider to be the "next generation of bone drugs" – namely, bone-targeting therapeutic agents that specifically coat bones immediately after systemic administration.

Idiotype Vaccine in Cancer Therapy

Ana María Vázquez, Head of Idiotype Vaccine Project, Center of Molecular Immunology, Havana, Cuba

Despite the many efforts dedicated to develop antitumor therapies, neoplasias are still one of the leading death causes all around the world. Active immunotherapy is one of the most promising fields in cancer research. Among the treatment strategies to develop an effective immune response against tumor-associated antigens is the use of anti-idiotype (Ab2) mAb as antigen surrogates. The use of Ab2 as vaccines arose as a consequence of Jerne's theory, which postulates that, due to the enormous potential diversity of immunoglobulin variable regions, the idiotype repertoire can mimic the universe of self and foreign epitopes. Advantage has been achieved

from the fact that they are easier to purify and scale up than the antigens they mimic and that they can functional mimic even non protein antigens. Anti-idiotypic vaccines directed to tumor-associated antigens have shown their capabilities to induce antitumor specific humoral and cellular responses, and in some cases a delayed tumor progression and an improved survival. Attractive targets for immunotherapy with anti-idiotype mAbs are Neu-glycolyl (NeuGc)-containing gangliosides, because these glycolipids are not normal components of the cytoplasmatic membrane in humans, but their expression has been demonstrated in several human malignant tumors. 1E10 mAb is an Ab2 murine mAb specific for an Ab1 mAb that reacts with NeuGc-containing gangliosides, sulfatides, and antigens expressed in some human tumors. In preclinical studies, this Ab2 was able to mimic NeuGc-containing gangliosides only in animals lacking expression of these Ags in normal tissues. Preparations containing 1E10 mAb were able to induce a strong metastatic effect in tumor-bearing mice. Different phase I clinical trials have been conducted in patients with advanced melanoma, breast cancer and lung cancer using aluminum hydroxide precipitated-1E10 mAb. The results of these clinical trials demonstrated the low toxicity and the high immunogenicity of this Ab2 vaccine. The induced anti-NeuGcGM3 antibodies recognized and directly killed tumor cells expressing the antigen, by a mechanism independent of complement activation and apoptosis, but that resemble an oncosis-like phenomenon. In the update analysis of a double blind, placebo controlled randomized clinical trial conducted in advanced NSCLC patients the intent to treat analyses showed an statistical significance benefice regarding overall survival for vaccine group. These results contribute to reinforce the therapeutic potential of anti-idiotypic vaccines and the importance of NeuGc containing gangliosides as antitumor targets.

Ana María Vázquez

Dr. Vázquez (BS in Biology, Havana University, PhD in Biological Sciences, Havana University, Cuba) is a senior researcher and. head of Idiotype Vaccine project. at the Center of Molecular Immunology in Havana. She is a Titular associate professor at the Department of Immunology of the Faculty of Biology at Havana University, member of the National PhD Thesis Examining Committee for Pharmaceutical Sciences, University of Havana and GRUM international associate member, Montreal, Canada.

Her research interests include the generation of monoclonal antibodies against non protein epitopes relevant for tumors and atherosclerosis, able to induce anti idiotype responses with therapeutic effects .and the study of the mechanisms mediating these responses. She has published over 55 articles in the field of monoclonal antibodies and has trained 15PhD students.

Dr Vázquez is member of the Oncology and Radiotherapy Cuban Society, the Immunology Cuban Society and member of Expert Vaccine Committee in Cuba.

Results of her scientific projects have deserved several National Award of Cuban Academy of Sciences (1988, 1995, 1996, 2001, 2005, 2006, and 2009) and the Scientific relevance award of Ministry of Science, Technology and Environment (2001). Besides, she received the recognition of the World Intellectual Property Organization on creativity and inventions in the field of monoclonal antibodies (2000). She received “Juan Tomás Roig” and "Carlos J. Finlay” medals for whole life research achievements.

Xenon Pharmaceuticals – A Genetics-based Drug Development Company

Conrad Winters, Senior Director, Compound Properties Group, Xenon Pharmaceuticals Inc., Burnaby, BC

There is a significant unmet medical need for a novel analgesic that is both effective and safe, as current treatment options leave many patients in ongoing pain or experiencing dose-limiting side effects such as nausea, vomiting, somnolence, gastrointestinal bleeding and cardiovascular complications.

To develop a novel analgesic, we have applied our clinical genetic approach to discover essential pain signaling pathways in humans by studying the genetics of the opposite phenotype; “absence of pain.” Knowing what genes are involved in human pain perception represents a key entry point into designing novel drugs to treat pain. Using our worldwide clinical network, we identified rare individuals with a congenital form of human analgesia known as Congenital Insensitivity to Pain (CIP). CIP is characterized by the total absence of the ability to perceive and/or understand any kind of noxious stimulus as painful. We discovered CIP

patients have mutations in the SCN9A gene resulting in absence of the $Na_v1.7$ sodium channel, thereby validating this drug target as essential for human pain sensation. Using a "Fast-to-Man" approach, we have advanced several analgesic products into clinical studies. This presentation will highlight some formulation considerations for these products. Oral XEN402 is in proof-of-concept trials using an inherited neuropathic painful syndrome due to over active $Na_v1.7$. Topical XEN402 is currently in Phase 2 trials for post herpetic neuralgia (PHN) and will be developed for painful conditions through a local action. A follow-on oral product, XEN403, is currently in Phase 1 trials.

Conrad Winters
Conrad Winters received his PhD in Pharmaceutics from the University of Bradford, UK in 1993 where he worked on cyclodextrin complexes under the guidance of Professor Peter York. He worked for Merck and Co. for 13 years in Canada and the U.S., working in preformulation, material science and formulation development roles on a number of projects that resulted in marketed products. He moved to biotech five years ago and is currently a Senior Director at Xenon Pharmaceuticals Inc. responsible for the Compound Properties Group. His current research interests include crystal doping, spray dried amorphous systems and hot melt extrusion formulations.

Making (Anti)sense: The Journey from University Laboratory, to Biotech Business to Clinical Proof of Concept

Mark Parry-Billings[1] & Paolo Renzi[2,] [1]Chiesi Pharmaceuticals, Parma, Italy, [2]Pulmonary Physician and Professor, University of Montreal, Montreal, QC

Antisense drugs are the most advanced class of the broad family of oligonucleotide-based therapeutics. They comprise a short single-stranded DNA with a complementary base sequence to a specific part of an mRNA coding for a targeted protein, whose expression is increased in the disease state of interest. Binding of the antisense to mRNA leads to knockdown of the synthetic machinery for the targeted protein, leading in turn to a therapeutic effect. There are a number of attributes of this therapeutic modality not least the specificity of the mechanism of action, significant potency and the ability to target the expression of any gene.

The application of antisense to the treatment of respiratory disease, notably asthma and COPD, was the focus of Topigen Pharmaceuticals Inc which was borne out of innovative research at the University of Montreal. The company was successful in securing a number of rounds of, primarily Canadian, venture capital financing which supported a number of antisense development programs leading to successful phase 2 clinical studies for an inhaled anti-inflammatory drug for the treatment of asthma. The company was sold to the Australian respiratory specialty pharmaceutical company, Pharmaxis, in 2010.

The journey of any technology and product development program and its transition from the university laboratory to a successful biotech business involves addressing a series of challenges. The correct strategy, in our experience, must address, in a practical manner, the focus of the R&D pipeline to ensure the correct balance between maximizing potential and delivering product development progress. Intellectual property must be robustly protected, development of the company's assets must be highly industrialized and executed to the appropriate quality standards and with a clear link to FDA regulatory process. Optimal financing over the life of the business is clearly central to success, as too is a judicious approach to partnering with pharmaceutical companies. The risk profile for Canadian biotech ventures remain high, with value being generated only by those with strong technology, significant financial backing and the ability to execute R&D efficiently and effectively,

Paolo Renzi
Dr. Paolo Renzi trained in internal medicine at University of Montreal and in respiratory diseases at Harvard. Since his return to Montreal in 1987 he has been working on the pathophysiology and therapy of airway diseases. D[r]. Renzi is a past National Researcher of the Fonds de Recherche en Santé du Québec (1990 to 2007), a pulmonary physician at Notre Dame Hospital and currently Professor at University of Montreal.

Dr. Renzi is internal or external reviewer for many journals, granting agencies, societies and government organizations. Dr. Renzi also consults as an expert in internal medicine, respiratory diseases or in pharmaceutical development. He is involved in provincial and national education programs and is also a member of pharmaceutical and biotech advisory boards. He has over 270

publications in peer reviewed journals, chapters and abstracts. He is also author or co-author of over 40 patents.

In between 1995 and 2007, Dr. Renzi founded Topigen Pharmaceuticals Inc and was either CSO, CEO or CMO of the company. Between April 2002 and July 2007, Topigen received in excess of $65,000,000.00 from various venture capital companies for the development of new therapeutic drugs for respiratory diseases, one of which is in Phase II studies in humans for asthma. Topigen is currently a subsidiary of Pharmaxis Australia.

Innovation in Rational Drug Product Formulation Development

Roch Thibert, Senior Director, Formulation Development, Corealis Pharma, Laval, QC

Pharmaceutical formulation requires a multitude of tools to assure the development of drug products with optimal characteristics such that they can deliver the therapeutic agent to its intended site of action. It is primordial to understand the active pharmaceutical ingredients' (API) physicochemical properties right from the initiation of the dosage form development activities. Nowadays, formulators are faced with very low solubility and poor permeability compounds. Thus, the presentation will provide an overview of the approaches available to the formulator to mitigate the risks inherent in the development of Biopharmaceutics Classification System (BCS) Class II and IV compounds. Tools such as classical size reduction or more novel nanomilling as well as rendering a molecule amorphous by spray drying, melt granulation, melt extrusion, and solubilisation by lipids or cyclodextrins will be described. Innovation will result from the unique combination and understanding of the physicochemical properties of the API, use of appropriate processing technology and the judicious selection of excipients to create the optimal dosage form.

Roch Thibert

Dr. Thibert has over 15 years of experience in the area of formulation research and development as well as clinical trial material manufacturing. He started his career as an Adjunct Professor at the Faculty of Pharmacy of the University of Montreal and later joined Merck Frosst Canada, ViroChem Pharma and, in 2009, Corealis Pharma. He is responsible for the formulation development of novel chemical entities as well as the GMP pilot plant for the manufacture of clinical trial material for Phase I to III human studies. He holds a Ph.D. in Pharmaceutical Science from the University of Montreal and has completed industrial post-doctoral studies.

Friday 11:00 AM - Track 3/4

Working with Industry

How can NSERC Partnership Programs Help Leverage Companies' R&D Investment Dollars

Pierre Bourassa, NSERC - Montreal

Opportunities and Obstacles in Transferring and Commercializing Innovation

Branka Barl, NRC-IRAP, Edmonton

NHP Company Perspective on the Value of Strong R&D for Company Growth & Success

NHP Industry Rep

Friday PM - Track 1

Pharmacogenomics (PGx)-Informed Therapeutics

Overview
Shinya Ito, Sickkids, & Bruce Carleton, University of British Columbia

Shinya Ito, MD, FRCPC
Dr. Ito is Professor and Head of the Division of Clinical Pharmacology & Toxicology, Hospital for Sick Children, Department of Paediatrics, University of Toronto. He is the principle investigator of a program of Paediatric Pharmacogenomics Medicine, called GeneMed, funded by the Ontario Ministry of Health and Long-Term Care. He is also a site director at the Toronto site of the Canadian Network of Pharmacogenomics for Drug Safety.

Warfarin: CYP2C9 and VKORC1.
Richard Kim, University of Western Ontario

[No abstract available]

Richard B. Kim, MD, FRCPC
Dr. Kim received his medical degree from University of Saskatchewan. After his internship and residency training, he went on to carry out Clinical Pharmacology fellowship training at Vanderbilt University and then stayed as a faculty member until 2006. He is currently Chair of the Division of Clinical Pharmacology and Professor of Medicine, Physiology & Pharmacology, and Oncology at the University of Western Ontario. He is also a Scientist and Director of the Centre for Clinical Investigation & Therapeutics (CCIT) at the Lawson Health Research Institute in London Ontario. The focus of his research has been elucidation of the factors that govern intersubject variation in drug responsiveness and the application of such findings to the delivery of Personalized Medicine.

Codeine: CYP2D6
Parvaz Madadi, Sickkids

[No abstract available]

Dr Parvaz Madadi
Dr. Parvaz Madadi is a Postdoctoral Research Fellow in the Division of Clinical Pharmacology and Toxicology at the Hospital for Sick Children in Toronto. She is also a Fellow of the Canadian Pharmacogenomics Network for Drug Safety and the Quebec Training Network for Perinatal Research. Dr. Madadi received a Doctorate in Clinical Pharmacology from the University of Western Ontario for her work on the pharmacogenetics of codeine in breastfeeding mothers under the supervision of Dr. Gideon Koren.

Azathioprine, 6MP: TPMT
Johann Hitzler, Sickkids

[No abstract or bio available]

Carbamazepine: HLA-B*1502
Michael Rieder, University of Western Ontario

[No abstract available]

Michael Rieder, MD, FRCPC
Dr. Rieder is a paediatric clinical pharmacologist at the University of Western Ontario. He qualified at the University of Saskatchewan and then trained at Wayne State University and the University of Toronto. He holds the CIHR-GSK Chair in Paediatric Clinical Pharmacology at the Schulich School of Medicine & Dentistry at the University of Western Ontario and is Chair of the Drug Therapy and Hazardous Substances Committee, Canadian Paediatric Society

Cisplatin: COMT, TPMT
Bruce Carleton, University of British Columbia

[No abstract or bio available]

Tamoxifen: CYP2D6
Ricardo Jimenez, University of British Columbia

[No abstract available]

Ricardo Jimenez
Dr. Ricardo Jimenez obtained his MD degree from Universidad La Salle, and his PhD in Pharmacology at the Center of Research and Advanced Studies, both in Mexico City. He has been a fellow at the University of Miami, as well as a research assistant at the National Institute of Public Health in Mexico. His former appointment was as a Medical Manager for Roche, where he developed and conducted clinical and epidemiology research in Latin America. Currently, he is a postdoctoral fellow at the University of British Columbia.

Beyond the HTA
Wendy Ungar, Sickkids

[No abstract available]

Wendy Ungar
Wendy Ungar MSc, PhD is a Senior Scientist in Child Health Evaluative Sciences at The Hospital for Sick Children, Toronto, Canada, an Associate Professor in Health Policy, Management and Evaluation, University of Toronto, and an Adjunct Scientist at the Institute for Clinical Evaluative Sciences. Dr. Ungar is the University of Toronto Program Director for the International Masters in Health Technology Assessment & Management and has held a Canadian Institutes of Health Research New Investigator career award. In 2010, Dr. Ungar's book, *Economic Evaluation in Child Health*, was published by Oxford University Press.

Dr. Ungar leads a program of research in the application of health economic methods to the paediatric population and also investigates the relationship between policies governing access to prescription medicines and health outcomes in children. In 2007 Dr. Ungar started TASK (Technology Assessment at Sick Kids), a health technology assessment unit focusing on technology assessment of pediatric health interventions (see: http://www.sickkids.ca/research/TASK/). Dr. Ungar and her research team created and maintain the PEDE database, a popular on-line health technology assessment tool for examining health economic evidence in children (see: http://pede.ccb.sickkids.ca/pede/search.jsp).

Poster Presentations
Day 1
Wednesday, May 25, 2011

CSPS Posters - Day 1

Wednesday, May 25, 2011

Biomedical Sciences

1. PGC-1α Transfection Restores Early Mitochondrial Functional Abnormalities in mdx Skeletal Muscle

Richard Godin[1], Frédéric Daussin[1], Alexis Ascah[1], Sonia Deschênes[1], Basil Petrof[2], Yan Burelle[1]. 1.Département de kinésiologie, Université de Montréal, Montreal, Quebec; 2.McGill University, Montreal, Quebec, Canada

Purpose: We examined the mitochondrial phenotype in skeletal muscle in the early phase of Duchenne muscular dystrophy (DMD) and, determined whether upregulation of mitochondrial biogenesis via PGC-1α transfection is beneficial in the mdx mice, a murine model of DMD.
Methods: PGC-1α plasmid transfection was conducted via electroporation in six weeks-old mdx and control mice. Seven days post-transfection, we characterized the mitochondrial phenotype with *in situ* assays.
Results: Compared to wild-type, mdx mice exhibited mitochondrial dysfunction including a lower oxidative capacity, a higher susceptibility to Ca^{2+}-induced PTP opening, and an adaptive increase in ROS buffering capacity. PGC-1α transfection largely restored mitochondrial density, as assessed by several marker proteins. Importantly, this translated into an increased mitochondrial Ca^{2+} buffering capacity and enhanced resistance to PTP opening.
Conclusion: Overall, this study reveals several mitochondrial functional abnormalities in the early phase of the disease, which were ameliorated 7 days after PGC-1α transfection. In particular, amelioration of mitochondrial Ca^{2+} buffering capacity may help to improve cellular Ca^{2+} regulation by limiting the adverse effect of excessive calcium levels, which characterizes DMD.

2. Active Immunization for Preventing Atherosclerosis Development

K Mellal[1*], V Brito[2*], S Giroux Portelance[1], D deBlois[1], H Ong[1], AM Vàzquez[2], S Marleau[1]. [1]Faculté de pharmacie, Université de Montréal, Montréal, QC, Canada; [2]Antibody Engineering Department, Center of Molecular Immunology, Havana, Cuba

Purpose: The pathogenesis of atherosclerosis is linked to the oxidation of low density lipoproteins (LDL) which is trapped in the extracellular matrix by an interaction with proteoglycans. Oxidized LDL (oxLDL) is then taken up by macrophage scavenger receptors leading to the formation of macrophage foam cells and fatty streaks. A mouse/human chimeric antibody (Ab) (chP3R99) which react against sulphated molecules such as glycosaminoglycans (GAG) was generated. We tested the hypothesis that active immunization with chP3R99 may induce an anti-idiotypic Ab cascade against GAG, thereby interfering with LDL binding to proteoglycans.
Methods: Apolipoprotein E (apoE)-deficient mice were fed with a HFHC diet containing 40% fat and 1.25% cholesterol from 4-week-old. HFHC-fed apoE-deficient mice have been immunized with 4 s.c. administrations of 50 mcg of chP3R99 at 2-week intervals.chP3S98 was used as a low reactivity Ab. Mice were sacrificed at 18 weeks of age, the aortas dissected from the aortic root to the iliac arteries for en face lesion analysis after staining with oil red-O. Reactivity of mouse sera against chP3R99, chP3S98, heparin, dermatan sulphate (DS), chondroitin sulphate (CS) and hyaluronic acid (HA) was assessed by ELISA.
Results: Our results show that immunisation with chP3R99 was associated with a 40% ($P < 0.01$) reduction in total aortic lesion areas. In contrast, administration of chP3S98 did not prevent atherosclerotic lesion development. Antiserum of mice immunized with chP3R99 showed high IgM and IgG reactivity against chP3R99, which remained

following antibody binding with an isotype-matched irrelevant Ab against the isotypic determinants of chP3R99. Antibody dilution curves showed lower reactivity of mouse sera against chP3S98. In addition, hyperimmune sera showed increased reactivity against heparin, DS, CS and HA.
Conclusion: The present study supports use of active immunization and the mounting of an idiotypic antibody network response against GAG side chains as novel approach to target atherosclerosis.
Supported by the Groupe de recherche universitaire sur le médicament (GRUM).
*Co-first authors

3. Vectors Targeting the Transferrin Receptor Label Brain Capillary Endothelial Cells after an Intravenous Injection

Sarah Paris-Robidas[1,2], Vincent Émond[1,2], Denis Soulet[2,3], Frédéric Calon[1,2]. [1]Faculty of Pharmacy, Laval University, Quebec (QC); [2]Centre Hospitalier de l'Université Laval (CHUL) Research Center, Quebec (QC); [3]Faculty of Medecine, Laval University, Quebec (QC), Canada

Purpose: The blood-brain barrier (BBB) considerably limits CNS drug development. The development of vectors crossing the BBB is thus a major challenge of CNS research. Transferrin receptors (TfR) are involved the transcytosis of their substrate transferrin through the BBB, and are thus being considered for brain drug targeting.
Methods: In the present study, we have characterized in vivo the brain distribution of two monoclonal antibodies (MAb) targeting the mouse TfR (Ri7 and 8D3) injected intravenously and compared to control IgGs. MAbs were fluorolabeled with either Alexa Fluor dyes 647 or 750. MAbs were injected intravenously and mice were sacrificed 1, 4 and/or 20h thereafter by intra-cardiac perfusion. Fluorescence was assessed on homogenates and sections.
Results: Intravenous injection of either Ri7 and 8D3 MAb coupled with Alexa Fluor 750 led to higher fluorescence emission in brain homogenates compared to control IgGs, indicating retention in the brain. Fluorescence microscopy analysis revealed that AF647-Ri7 signal was confined to brain cerebrovasculature, colocalizing with an antibody against collagen IV, a marker of basal lamina. Confocal microscopy analysis confirmed the delivery of injected Ri7 MAb into brain endothelial cells using the pericyte marker anti-α-smooth muscle actin (α-SMA), the endothelial marker CD31 and the collagen IV antibody. Although immunohistochemical data on mouse brain sections confirmed the presence of TfR on both microvessel endothelial cells and neurons, systemically injected AF647-Ri7 did not reached neurons or astrocytes as identified using antibodies specific for neuronal nuclei (NeuN), or glial fibrillary acidic protein (GFAP), respectively.
Conclusion: Our data show that anti-TfR vectors injected intravenously readily accumulate into brain capillary endothelial cells, thus displaying strong drug targeting potential.

4. New Therapeutic Opportunities for the IL-1 Receptor Modulator 101.10

Christiane Quiniou, Frank Cloutier, Kim Beauregard, Xin Hou, William Lubell and Sylvain Chemtob. University of Montreal, CHU Sainte-Justine, Montréal QC

Background: IL-1 receptor is involved in diseases wherein inflammation plays a major role. Previous studies in our and other laboratories have shown that the modulator of interleukin (IL)-1 receptor named 101.10 is effective *in vivo* in inflammatory bowel disease and hypoxic-ischemic brain injury. The extent of the efficacy of 101.10 has not been sufficiently investigated, particularly as it applies to CIA. Moreover, structure-activity relationship of 101.10 has yet to be explored.
Objective: a) Test the efficacy of 101.10 in an animal model of rheumatoid arthritis, namely collagen induced arthritis (CIA). b) Begin to determine structure-activity relationship of 101.10, through development of analogues.
Methods: CIA was generated using bovine type-2 collagen (CII) (2 mg/ml) and Freund's adjuvant (4 mg/ml). Upon the first clinical signs of joint inflammation, mice were randomized to vehicle (n=7) or treatment with 101.10 (10 mg/Kg, i.p. twice daily) (n=7) for 30 days. Bone damages and inflammatory response were evaluated by X-rays and histology, respectively. Peptide analogues (L and D isomers) were tested in IL-1-induced hyperthermia. □ and □□lactam derivatives of 101.10 were also synthesized and the activity of these compounds were tested in vitro on IL-1-induced TF-1 proliferation (Cyquant NF,

Invitrogen).

Results: 101.10 diminished major clinical signs of arthritis, notably joint swelling, redness and deformities, detected visually and radiographically. Likewise, histological evaluation revealed that 101.10 preserved synovial and bone integrity compared to vehicle-treated animals. Correspondingly, fewer joints were affected in animals treated with 101.10. Based on reproducible efficacy of 101.10 in models of major inflammatory conditions, peptide analogues were generated. The analogue 2516-3 could prevent 50% of IL-1-induced hyperthermia, but not 2516-2 and 2516-4. Accordingly, small peptidomimetics were developed and essential structural motifs for activity identified based on *in vitro* assays. Examination of and *β*-Amino *γ* -lactam (Agl and Bgl) derivatives of 101.10 for their potential to inhibit IL-1-induced thymocyte cell proliferation using a novel fluorescence assay revealed that certain analogs exhibited retained and improved potency relative to the parent peptide 101.10 by (100 % efficacy of inhibition of proliferation).

Conclusion: Our results show that 101.10 is effective *in vivo* in different IL-1-dependent inflammatory conditions. Findings are substantiated by *in vitro* and *in vivo* efficacy of 101.10 derivatives. The data provide new perspectives in drug development targeting IL-1, without the drawbacks of large biologics and prolonged immuno-suppression associated with currently available Fc-containing compounds.

5. Hyaluronic Acid Signals for Repair in Ethanol-induced Apoptosis in Cultured Skin Cells

Manuela G. Neuman, Loida Oruña, Gabriel Coto, Radu Nanau, Marc Vincent. Departments of Clinical Pharmacology & Toxicology, University of Toronto, *In Vitro* Drug Safety, MaRS Discovery District, Toronto, Ontario, Canada

Ethanol is commonly used in cosmetic and pharmaceutical preparations. The present study aimed to assess ethanol-induced apoptosis and the possible repair by hyaluronic acid (HA) in *in vitro* models. We also aimed to determine the modulation of pro-inflammatory cytokines tumor necrosis factor alpha (TNF-) and interferong gamma (IFN-) production and their release, when A431 epidermoid skin cells and mouse fibroblasts were exposed to different concentrations of ethanol. Another objective of this study was to determine if HA could prevent ethanol-induced skin toxicity. We treated human A431 epidermoid skin cells and mouse fibroblasts with two concentrations of ethanol for 24 hours. HA obtained from umbilical cord excision was used at three concentration levels to determine its efficacy in the treatment.

Results: Treatment of cells with ethanol at 50 mM and 100 mM increased both the percentage of cells undergoing apoptosis, as well as the release of tumor necrosis factor-alpha (TNF-α) into the culture medium. Treatment with 50 mM ethanol was only slightly toxic to mouse fibroblasts (6% ± 2.2%) and to A431 cells (8% ± 2.5%). Exposure to 100 mM ethanol for 24 h resulted in higher cytotoxicity compared with findings for control treatment (mouse fibroblasts 24% ± 7.2%; A431 cells 18% ± 2.5%; $P<0.001$ vs. control and $P<0.05$ vs. 50 mM ethanol). HA 2% reduced the apoptosis due to 50 mM ethanol-exposure in A431 cells (8% ± 2.5% to 4% ± 1%; $P<0.001$). HA 4% further reduced the cytotoxicity (4% ± 1% to 2% ± 0.5%; $P<0.05$ vs. HA 2% treatment). HA 8% had no effect on cytotoxicity. The concentrations of TNF- and IFN- in the medium of cells treated with 100 mM ethanol for 24 h were significantly different from those of control cells, as explained in the table captioning. HA 2% significantly lower these concentrations. Examining by electron microscopy, treatment of A431 cells with 100 mM ethanol for 24 hours caused disruption of tight junctions, ballooning of the endoplasmic reticulum and alterations in the size and shape of mitochondria. Many cells treated with 100 mM ethanol underwent apoptosis. Cells exposed to ethanol and 2% HA were more regular, with tight junctions and normal looking mitochondria.

In conclusion, ethanol induces apoptosis in skin cells by enhancing the effects of TNF-α and IFN- HA in the 2% and 4% concentrations reduced inflammation both in human A431 epidermoid skin cells and in mouse fibroblasts.

6. Microperoxidase-11 Inhibition by 4-aminobenzoic Acid Hydrazide: A Free-Radical Metabolite Induced Inhibition

Pratik Arvadia[1], Malyaj Narwaley[1], Randy Whittal[2], Arno G. Siraki[1]. Faculty of Pharmacy and Pharmaceutical Sciences[1], Department of Chemistry

Mass Spectrometry Facility[2], University of Alberta, Edmonton, AB, Canada

Purpose - 4-aminobenzoic acid (4-ABAH) is a well known irreversible peroxidase inhibitor. The exact mechanism of peroxidase inhibition by hydrazide is still to be determined. Microperoxidase-11(MP11) is a heme containing undecapeptide obtained from cytochrome c oxidase simulates peroxidation reactions. MP11 being relatively small protein as compared to other peroxidases makes analytically suitable as a model system. The main aim of study is to determine if MP11 is inhibited by 4-ABAH and if the free-radical metabolites generated, are involved in this inhibition.
Methods - 4-ABAH mediated MP-11 heme inhibition was studied spectrophotometrically. The effect of 4-ABAH on peroxidase activity of MP-11 was determined by the guaiacol assay. 4-ABAH free-radicals generated in the presence of MP-11 were characterized by spin-trapping with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) in Electron spin resonance (ESR). To detect spin adducts, western blotting was performed. The confirmation of 4-ABAH adduct with MP-11 was further determined by MALDI-TOF MS and FTICR-MS analysis.
Results: MP-11 irreversible inhibition by 4-ABAH was observed with a characteristic shift in the visible spectrum. Moreover, the presence of 4-ABAH in the sample had significant inhibition of peroxidase activity of MP-11 as when compared to preparations containing DMPO. C-centred benzoyl radicals were detected in ESR in presence of MP11 as when compared to other controls. The adduct formation between MP-11 and 4-ABAH was observed in western blots. In addition, these covalent adduct of MP11 and benzoyl radical of 4-ABAH was detected through MALDI-TOF MS and further FTICR results confirmed the elemental mass of these adducts.
Conclusion: 4-ABAH proves to be a specific peroxidase inhibitory substrate for MP11 by inhibiting heme and peroxidase activity of the enzyme. Moreover, 4-ABAH is metabolised to a free-radical metabolite that leads to the enzyme inactivation. Further, the presence of oxidants like H_2O_2 increases the generation of free-radicals whereas the presence of DMPO scavenges the free-radical generated and thereby decreasing their amount in the system.

7. Surface Functionalized Single Walled Carbon Nanotubes for Tumor Specific Targeting – Characterization and *in vitro* Studies

Raja Chemali, Meenakshi Malhotra, Catherine Tomaro-Duchesneau, Shyamali Saha, Imen Kahouli, Chris Jackson, Hani Al-Salami and Satya Prakash. Biomedical Technology and Cell Therapy Research Laboratory, Departments of Biomedical Engineering, Physiology, and Artificial Cells and Organs Research Center, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

Purpose: Cancer is a major health concern and its prevalence has been on the rise worldwide. Traditional cancer drug delivery and chemotherapy demonstrates significant side-effects which need to be overcome. The absence of therapeutic specificity and their failure to reach the targeted tissue are some challenges which remain to be addressed. With the unique physical and chemical properties of biotherapeutic, functionalized single walled carbon nanotubes (f-SWNT), these have shown promising potential in biomedical applications, including tumor targeting. The high surface area of SWNTs makes them efficient drug carriers into tumor tissues which require high drug dose uptake for any therapeutic effect. In this study we are investigating the cellular uptake and cytotoxicity of different dilutions of functionalized SWNT.
Methods: MCF-7 and SW-480 cells were used to investigate the therapeutic uptake and toxicity of a number of different single walled carbon nanotube formulations (SWNTs). All of the SWNTs formulations were tagged with the detection molecule, fluorescein isothiocyanate (FITC). Polyethylene glycol (PL-PEG2000) was used and functionalized on SWNTs in order to increase their solubility in aqueous solutions. The functionalized SWNTs were further conjugated with ligands to increase tumor specific targeting and to decrease non-specific therapeutic uptake. The two cell lines were treated and incubated with various formulations and the data was analyzed for both cytotoxicity and uptake over a period of 48 hours. Fluorometry was used for measuring the uptake of the formulations, and an MTS assay was used for measuring citotoxicity, both at 490 nm using a multiplate reader.
Results: Ligands were successfully conjugated on SWNTs, as confirmed by ^{1}HNMR and FTIR. The functionalized SWNTs formulations demonstrated a considerable cellular uptake as confirmed by

fluorometry. In fact, the formulations uptake was five times higher than the uptake of unfunctionalized carbon nanotubes (negative control) following 48 hours of incubation with the cell line. The toxicity profile showed no significant cell apoptosis following the 48 hour treatment period.
Conclusion: Functionalized SWNTs can potentially be used as a targeted drug delivery carrier. These formulations will increase drug delivery.
Acknowledgement: CIHR research operating grant. MOP 93641 to Dr. Satya Prakash.

8. The Stability of Bile Salt Hydrolase Producing Microencapsulated *Lactobacillus* Bacteria

Daniel Marinescu, Hani Al-Salami, Catherine Tomaro-Duchesneau, Satya Prakash*. Biomedical Technology and Cell Therapy Research Laboratory, Departments of Biomedical Engineering, Physiology, and Artificial Cells and Organs Research Center, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

Purpose: Probiotics are dietary supplements containing bacteria which, when administered in adequate amounts, confer a health benefit on the host. Probiotics have been shown to be beneficial in a wide range of conditions including infections, allergies, and metabolic disorders (1). The process of developing probiotic therapeutics involves *in-vitro* and *in-vivo* studies to characterize the pharmacological formulation for efficacy, safety, stability and other features. Recently, probiotic bacteria which express the bile salt hydrolase enzyme (BSH) have been the subject of growing interest, due to their potential therapeutic applications (2). Bile is a complex fluid containing water, electrolytes and other organic molecules including bile acids, cholesterol, phospholipids and bilirubin that flows from the bile duct into the small intestine. BSH hydrolyses bile acids and is expressed by certain bacteria as a protective mechanism against bile acids antibacterial activities (3). However, increasing BSH expression in the gut should increase bile acids degradation; resulting in more utilization of body cholesterol. The administration of BSH and BSH-active probiotics has indeed been linked with the reduction of cholesterol and triglycerides.
Methods: Bacterial viability under different conditions and the amount of BSH secreted by *Lactobacillus reuteri* were respectively analyzed *in vitro* using agar-plates and a developed HPLC method. HPLC was used to detect the loss in bile acid substrate (in flasks), through a simple ninhydrin-based analysis which detects the increase in glycine from glycodeoxycholic acid substrate. Following microencapsulation within APA-microcapsules, stability was monitored via microscopy at different stages of GI transit using a simulated GI model.
Results: *Lactobacillus reuteri* showed good BSH activities under different physiological conditions while maintaining strong viability and microcapsule stability within APA (alginate-polyLysine-alginate) microcapsules. Specifically, an activity of 5.96 ± 0.35 µmol of GDCA/hr/g of microcapsules was measured. Following an MRS-acid challenge, the BSH activity of probiotics was increased to 10.16 ± 0.46 µmol/hr/g. Throughout, a viability above 109 cfu/ml after 2h of incubation at pH 3.0 was maintained for the strain. The microcapsule's membrane integrity showed optimal stability at a diameter of 619 ± 31 µm. Following simulated stomach transit, microcapsules remained intact; with <5% deformation and a reduction in diameter to 564 ± 58 µm due to core shrinkage. Following simulated intestinal exposure, between 85-90% of microcapsules remained intact and showed swelling; increasing in diameter to 731 ± 72 µm.
Conclusion: Since APA microencapsulation is known to protect probiotics during gastric transit. This property becomes relevant for the use of these bacteria in oral therapy. These stability studies provide good support for the maintenance, via APA-microencapsulation, of the probiotics' viability under strenuous physiological conditions.
Acknowledgement: The authors would like to acknowledge the financial support from Micropharma Ltd.

9. Characterization of Exosomes Derived from Different Prostate Cancer Cell Lines

Elham Hosseini-Beheshti*, Steven Pham, Hans Adomat, Emma S. (Tomlinson) Guns. The Vancouver Prostate Centre, University of British Columbia, Vancouver, BC Canada.

Introduction: Exosomes are extracellular nano-vesicles which are secreted upon fusion of multivesicular bodies with the plasma membrane. They have been shown to be derived from a

multitude of cell types and, while it is postulated that they are important for membrane trafficking as communication vesicles, their relevance in cancer and specifically prostate tumour growth and progression has yet to be determined. Factors such as stage of cancer, cell type and cell cycle could affect the amount and composition of exosomes formed and secreted. While the mechanisms underlying exosome formation and secretion are not fully understood, proteomic classifications and lipid analysis of exosomes derived from different cell lines indicate many common membrane and cytosolic protein/lipid markers. Additionally differential protein content reflects a prognostic potential. Other studies on tumour-related microvesicles suggest the role of these selectively enriched exosomes in cancer progression. The presence of differential protein markers in and on these entities may provide potential as biomarkers during cancer diagnosis.

The specific aims of this study are: i) To characterize exosomes derived from different prostate cancer cells, and ii) To delineate the role of specific proteins/lipids present within exosomes which are involved in prostate cancer progression.

Methods: Exosomes were purified from culture media of PC3, DU145, VCaP, C4-2, LNCaP and RWPE-1 cells cultured in serum free media for 72 hours at 37°C. Purified exosomes were analyzed by western blot using different exosome markers including (Actin, Tubulin, HSP-70, HSP-90, LAMP2, Rab 5, CD 9) in all six different cell lines. In addition, isolated exosomes were negatively stained on formvar-coated carbon EM grids for transmission electron microscopy imaging. Furthermore the protein and lipid profile of exosomes were characterized using liquid chromatography -Mass spectrometry.

Results: We confirmed that exosomes are released from a range of androgen sensitive and insensitive prostate cancer cell lines. Using Western Blot analysis alongside Transmission Electron Microscopy imaging, we confirmed these secreted vesicles are in fact exosomes. Proteomic and lipid analysis of exosomes derived from prostate cancer cells has been conducted. Proteomic analysis of exosomes derived from these different prostate cancer cells lines versus normal epithelial prostate cell reveals insightful information about protein classification which is likely to have resulted from a sorting mechanism.

Conclusion: This study highlights a potential of differential protein composition of exosomes as a source of diagnostic biomarkers for prostate cancer via non-invasive testing.

10. Effect of Lactobacillus Acidophilus on SW 480 Colon Adenocarcinoma Cell Proliferation: In Vitro Analysis

Imen Kahouli, Catherine Tomaro-Duchesneau, Meenkashi Malhotra, Hani Al-Salami, Shyamali Saha, Laëtitia Sonia Rodes, Marc Fakhoury, Raja Chemali, Satya Prakash*. Biomedical Technology and Cell Therapy Research Laboratory, Department of Experimental Medicine, Biomedical Engineering and Artificial Cells and Organs Research Centre, Faculty of Medicine, University McGill, Montréal, QC

Introduction: Colon cancer is a prominent disease, listed as the leading cause of cancer mortality throughout the world, according to the WHO. Probiotics, including lactic acid bacteria (LAB), are living microorganisms that affect the host in a beneficial manner by improving nutritional and microbial balance in the intestinal tract. Interestingly, several researchers have studied the anti-tumor effects exerted by LABdf. In fact, recent in vitro and in vivo research has demonstrated the anti-tumor effects of lactobacilli on the integrity of the gastrointestinal epithelium. Lactobacillus acidophilus has been shown some beneficial activities in numerous animal and in vitro models, but their mechanism of action, in terms of inhibiting colon cellular proliferation and in interfering the neoplastic transformation of gastrointestinal mucosa, remains to be demonstrated. The goal of this research is to evaluate the in vitro effects of Lactobacillus acidophilus cells on the proliferation of human colon cancer in vitro and for this we used SW480 cell line. In order to determine the inhibition of the cancer cell growth and proliferation, a probiotic formulation was prepared. After the incubation of the cancer cell with different concentration of the probiotic treatments, the assessment of cell proliferation is determined with by the evaluation of cell growth and apoptosis by luminescence and other viability/apoptosis methods.

Results showed that Lactobacillus acidophilus probiotic formulations affected the neoplastic epithelial cells by decreasing cell viability and proliferation specially the first 24 hours. In fact, after 12hrs, cancer cell viability treated with 1:1 and 1:2 concentration doses of the probiotic treatment

decreased, respectively, to 41% and 76% and apoptosis increased, respectively, to about 70% and 40%. Then, after 24hrs of incubation with the probiotic treatment at 1:1 and 1:2 concentration doses, respectively, SW480 cancer cell viability decreased to 79% and 97% while apoptosis was at 23% and 9% , compared to non-treated cell at each time point. Cell proliferation and differentiation into cancer cells was inhibited be the reduction of cellular products. These results indicate that Lactobacillus acidophilus metabolic activity is responsible for in vitro inhibition and proliferation and cancer cells.

Keywords: Cell proliferation; polyamine, apoptosis, colon cancer; Lactobacillus Acidophilus, probiotic; tumor cell lines, bacterial extract

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11. Black Spruce Polyphenols: Chemical Characterization and Study of their Effects on the IL-8 Production in Normal and Psoriatic Keratinocytes Stimulated with TNF-α

Martha-Estrella García-Pérez[1,2], Mariana Royer[2], Daniel Rusu[3], Patrice E Poubelle[3], Tatjana Stevanovic[2], Roxane Pouliot[1]. [1]Centre LOEX de l'Université Laval, Génie tissulaire et régénération: LOEX - Centre de recherche FRSQ du Centre hospitalier affilié universitaire de Québec and Faculté de Pharmacie, Université Laval, Québec, QC; [2]Centre de Recherche sur le Bois, Département des sciences du bois et de la forêt, Faculté de foresterie et géomatique, Université Laval, Québec, QC, [3]Centre de Recherche en Rhumatologie et Immunologie. Département de médicine, Faculté de Médicine. Université Laval, Québec, QC, Canada

Purpose: Polyphenols are multifunctional molecules that could be used for the treatment of multi-causal diseases, such as psoriasis, a skin disorder affecting 2% of the world's population. This work aimed: a) to identify the polyphenols present in black spruce (BS) (Picea mariana) bark aqueous extract b) to determine their toxicity on normal human keratinocytes (NHK) c) to determine their effects on the IL-8 production in normal and psoriatic keratinocytes (PK) stimulated with TNF-α.

Methods: Polyphenols from BS were obtained by hot water extraction and further extracted with ethyl acetate (EAc). After removal of solvent and lyophilisation, the EAc fraction was dissolved in water and partitioned with dichloroethane (DE). Purification of the DE fraction by flash chromatography and RP-HPLC allowed the characterization of fourteen molecules by ^{1}H and ^{13}C NMR spectral data analysis. The toxicity of the EAc fraction (100-500 µg/ml) on NHK incubated during 24 and 48 h was evaluated by the lactate deshydrogenase (LDH) release and the propidium iodide (PI) staining. The effect of this fraction (compared to the standardized French maritime pine bark extract Oligopin® and Dexamethasone) on the production of IL-8 by TNF-α-stimulated NHK and PK was evaluated by ELISA.

Results: Fourteen compounds were identified from DE fraction, most of them belonging to lignans: cedrusin (**1**), isolariciresinol (**2**), (2R,3S)-secoisolariciresinol (**3**), 7(S)-hydroxymatairesinol (**4**), 7(R)-hydroxymatairesinol (**5**), 1,4-Benzodioxin-6-propanol,2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-, (2S, 3S)-(trans) (**6**), pinoresinol (**7**) and epi-pinoresinol (**8**). The EAc fraction showed no toxicity at the dose levels tested. This fraction (100-500µg/ml) significantly suppressed the TNF-α induced IL-8 production by NHK and PK, with 38-100 and 36-100 % inhibition respectively.

Conclusion: The results obtained in this study indicate that black spruce polyphenols from the EAc fraction are potent inhibitors of the TNF-α induced production of IL-8 in normal and psoriatic keratinocytes in vitro. Chemical characterization of this extract demonstrated that Picea mariana bark is particularly rich in lignans, which were identified for the first time in the bark of this specie.

12. Dietary Supplementation with Phytosterol and Ascorbic Acid Reduces Mass Accumulation in High Fat-fed Mice

Ian T. Y. Wong, Rachel Neumann, Sheila J. Thornton and Kishor M. Wasan. Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC

Background: Dietary supplementation with phytosterols is known to reduce elevated plasma cholesterol levels, a risk factor in the development of atherosclerotic lesions. As atherosclerosis is an inflammatory disorder, administration of antioxidants such as ascorbic acid is believed to lead

to a reduction in the risk of atherosclerotic cardiovascular disease. Supplementation with disodium ascorbyl phytostanyl phosphate (DAPP), a phytostanol linked to ascorbic acid by a phosphodiester bond, resulted in reduced plasma cholesterol and decreased atherosclerotic lesion formation in high fat-fed mice. Unexpectedly, animals on the DAPP supplement also exhibited significant reduction in mass accumulation. Phytosterols or ascorbic acid alone has also been shown to reduce mass accumulation; however, to date, no mechanism has been identified. To investigate potential mechanisms, we examined the effect of phytosterol (PS) and ascorbic acid (AA) supplementation, both alone and in combination (PSAA), in high fat-fed mice.

Methods: Animals were fed a high-fat (HF) diet with or without supplements of 1% w/w AA, PS, or PSAA thereof for 18 weeks; mass, food and water intake were assessed weekly for the first 12 weeks. Resting metabolic rate (RMR), maximal oxygen consumption (VO2 max), food transit time, fecal output were measured and plasma and fecal lipids, cholesterol, triglycerides and non-esterified fatty acids were assessed. To characterize the acute effect of the diets on food transit time and fecal output, age-matched mice were exposed to the diets for 72 hours.

Results: Mice fed a HF diet supplemented with PSAA showed a reduction in mass accumulation over the 18 week study when compared to all other groups. No difference in food intake, water intake, RMR, VO2 max or metabolic scope was observed between any of the experimental diets and control. After chronic exposure to supplements, the PS and PSAA groups exhibited an increased passage rate and decreased fecal output after a 72 hour exposure to a HF diet.

Conclusion: Supplementation with either PS or AA did not result in a reduction of mass accumulation; however, when the diet contains both supplements (PSAA), a significant decrease in mass accumulation occurred. This could not be attributed to differences in food intake or metabolic rate between the groups. When exposed to a HF diet, the PS groups exhibited a decrease in food transit time and fecal output, suggesting that the intestine may upregulate surface area and/or transporter activity in response to dietary PS. This upregulation may alter AA absorption and potentiate mass loss in the PSAA group.

Acknowledgements: Funding for this project was provided by CIHR. Ian Wong is an awardee of the National Summer Student Research Program Awards to Present Research Findings sponsored by GlaxoSmithKline Inc.

Pharmaceutical & Analytical Chemistry

13. Quantitative Determination of Alendronate Sodium in Human Plasma using a Validated LC/MS/MS Method: Application to Clinical Pharmacokinetic Studies

Marie-Helene Vallee, Mohamed Khalil Tamim, Fethi Trabelsi, and Mario Tanguay. PharmaNet Canada, Inc.

Purpose: Alendronate, an aminobiphosphonate, is a specific inhibitor of osteoclast-mediated bone resorption. Its bioavailability is limited by poor absorption due to its highly polar structure. To date, detection of alendronate in human plasma as well as pharmacokinetic profiles has been challenging as opposed to detection in urine. The aim of the present study is to describe the bioavailability of alendronate under fasting conditions using a novel sensitive and specific LC-MS/MS method in human plasma developed at PharmaNet Canada, Inc.

Methods: The present open-label randomized, 2-period, 2-sequence bioavailability study was carried out at PharmaNet Canada, Inc. (Quebec, CANADA). Twelve healthy adult volunteers were randomly assigned to receive either single 40 mg or 70 mg dose of alendronate sodium (Fosamax®) followed by a 10-day washout period. Alendronate was administered after 10 hours overnight fast and adverse events were monitored by PharmaNet clinical staff. Plasma alendronate sodium concentrations were determined using a validated LC-MS/MS method with a calibration curve range of 0.05-50 ng/mL. Pharmacokinetic properties, including $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, C_{max}, T_{max} and $T_{1/2}$, were determined using noncompartmental analysis.

Results: Following oral administration of either 40 mg or 70 mg alendronate to healthy adult volunteers, observed C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$ were respectively: 9.21 ± 5.33 to 23.08 ± 14.67 ng/mL, 29.14 ± 19.69 to 57.02 ± 33.74 ng.h/mL, and 30.68 ± 20.93 to 59.51 ± 35.10 ng.h/mL. The T_{max} and $T_{1/2}$ were found to be around (1.32h; 4.18h) and (1.17 h; 4.30h) for both doses respectively.

Conclusion: To our knowledge, this is the first

description of the pharmacokinetic parameters of alendronate sodium determined by LC-MS/MS in human. Results from this pilot study suggest that clinical pharmacokinetic studies (including bioequivalence and bioavailability studies) are feasible with alendronate data in plasma.
Presented at the AAPS 2010 meeting held on November 15, 2010.

14. Simultaneous Determination of a Novel Antitrypanosomal Compound (OSU-36) and its Ester Derivative (OSU-40) in Plasma by HPLC: Application to First Pharmacokinetic Study in Rats

Pavel Gershkovich[1], Kishor M. Wasan[1], Olena Sivak[1], Summer Lysakowski[1], Carolyn Reid[2], Kokku Premalatha[2], and Karl A. Werbovetz[2]. [1]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada; [2]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, USA.

Purpose. To develop an HPLC-UV method for determination of a novel antitrypanosomal compound (OSU-36) and its ester prodrug (OSU-40) in rat plasma and to apply the method for pharmacokinetic evaluation of both compounds in rats.
Methods. Since OSU-36 and OSU-40 were not stable in plasma resulting in highly non-linear calibration curves and poor sensitivity, the plasma samples were stabilized using paraoxon and ascorbic acid. The sample treatment included protein precipitation by acetonitrile; evaporation; reconstitution with acetonitrile and filtration. The chromatography conditions included Xterra RP18 3.5µm 4.6X100mm column and gradient mobile phase system of acetonitrile-water.
Results. The limits of quantification (LOQ) using 200 µL of plasma sample were 50 ng/mL and 40 ng/mL for OSU-36 and OSU-40, respectively. The intra- and interday precision and accuracies were below 13% for low, medium and high concentration quality control samples for both compounds. While OSU-40 has been stable in all tested handling conditions, OSU-36 was unstable in plasma after 20 days storage at -80°C or at 4h 25°C storage. The developed method has been applied for a pharmacokinetic study in rats which revealed that an ester prodrug OSU-40 is rapidly converted to OSU-36 within the plasma compartment by plasma esterases. OSU-36, in turn, relatively quickly undergoes oxidative metabolism, including within the plasma compartment.
Conclusions. A supplementation of rat plasma with an esterase inhibitor to prevent degradation of ester prodrug (OSU-40), and with antioxidant to prevent oxidation of OSU-36, is necessary for reliable determination of both compounds. Due to limited stability of OSU-36 even in stabilized rat plasma, long-term storage of samples or prolonged handling in room temperature conditions is not recommended.
Acknowledgement: This work was supported by Canadian Institutes of Health Research (CIHR) and Neglected Global Diseases Initiative of University of British Columbia (NGDI-UBC).

15. Novel NSAIDs Prodrugs with Improved Anti-Inflammatory Activity, Low Ulcerogenicity and Potential Chemopreventive Properties: Do We Really Need a Nitric-Oxide Releasing Group?

Sarthak Jain, Mohamed A.M. El Gendy, Ayman O. El-Kadi, Carlos A. Velázquez. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Purpose: Our study was aimed to develop and evaluate the anti-inflammatory activity and ulcerogenicity of new hybrid NO-NSAIDs possessing three polyphenols (tyrosol, 3-hydroxybenzyl alcohol (3-HBA), or 4-hydroxybenzyl alcohol (4-HBA)) as linkers between the carboxylic acid group of NSAID (Aspirin, Iboprofen and Indomethacin) and the nitric oxide-releasing moiety (*N-diazen-1-ium-1,2-diolate).*
Methods: Ester prodrugs were formed by simple nucleophilic displacement reactions between tyrosol, 3-HBA, or 4-HBA with the corresponding NSAIDs acid chloride dissolved in tetrahydrofuran (THF).
Results: As part of our project, we screened these intermediate prodrugs for anti-inflammatory activity *in vitro (COX-1 and COX-2 immunoassay), in vivo (carrageenan-induced rat paw edema assay), ulcerogenicity (Ulcer Index Assay), MTT assay, and determination of Nqo1 activity.* Selective COX–2 inhibition for Ibuprofen and Indomethacin prodrugs was observed with a selectivity index ranging from 32220 to 21.69 in COX-1 & COX-2 immunoassay. At equimolar doses (p.o.) prodrugs showed enhanced *in vivo* anti-inflammatory activity (28-

85%) compared to their parents. The *in vivo* ulcer index assay gave remarkable results, as most prodrugs were non toxic as compared to their parents. MTT assay was performed in HepG2 cells to determine the optimal concentrations that can be used in cell based study. Further capability of these drugs was tested for inducing Nqo1 phase II enzyme. They increased activity of Nqo1 enzyme by 142% to 225% as compared to control (100%).
Conclusion: This study reveals that i) a nitric oxide-releasing moiety is not required to prevent the ulcerogenic properties of NSAIDs, and ii) only alcohols with anti-oxidant properties are suitable for the design of effective and safe NSAID prodrugs. This study offers evidence to propose the design of effective NSAID ester prodrugs *without* a complex nitric oxide-releasing group, but use of anti-oxidant polyphenols instead. The results showed us that simple NSAID prodrugs are a suitable and convenient alternative to the use of NSAIDs for the long-term treatment of inflammation, pain, and cancer chemoprevention.

16. Design, Synthesis, and Preliminary Evaluation of Resveratrol Derivatives Possessing a Salicylate/Acetylsalicylate Moiety as Potential DNMT-1 Inhibitors for the Chemoprevention of Colorectal Cancer

Fahad Aldawsari[1], Praveen P. N. Rao[2], Jose Medina[3] and Carlos Velázquez[1]. [1]Pharmaceutical Sciences, University of Alberta, Edmonton, AB; [2]School of Pharmacy, University of Waterloo, Kitchener, ON, Canada; [3] Torrey Pines Institute for Molecular Studies, Port St Lucie, Florida, USA.

Background: Resveratrol is a natural polyphenol with a wide variety of biological applications, including anti-oxidant, anti-inflammatory, and chemopreventive.
Purpose: The goal of our research project is to study the structure-activity relationships of a new group of aspirin-like resveratrol derivatives possessing either a salicylate or an acetylsalicylate moiety, as new dual anti-inflammatory/chemopreventive agents.
Methods: Wittig reaction was employed to synthesize the resveratrol analogous. Phosphonium salts 3,5-dimethoxybenzyltriphenyl phosphonium bromide and 4-Methoxybenzyltriphenyl phosphonium bromide were used as source of ylides.
Results: 1) We were able to synthesize methoxylated resveratrol (reference compound) and four new resveratrol derivatives: 3,4',5- trimethoxy-3'-carboxylate methyl ester stilbene, 4,4'-Dimethoxy-3'-carboxylate methyl ester stilbene, 3,5-dimethoxy-4'-acetoxy-3'-Carboxylate methyl ester stilbene and 4-methoxy-4'-acetoxy-3'-carboxylate methyl ester stilbene; 2) We observed favorable molecular interactions (docking studies) between resveratrol derivatives and the active site of COX-1, COX-2, and DNMT-1. In this regard, salicylate-like resveratrol analogues adopt a suitable conformation favoring the formation of hydrogen bonds with Glu^{1265} and $Arg^{1311,}$ which are key aminoacid residues that participate in the catalytic mechanism of DNA methylation by the DNMT-1 active site.
Ongoing work: We are currently evaluating the anti-inflammatory profile of salicylate-like resveratrol analogues *in vitro* (COX-1, COX-2, and 5-LOX inhibition), and *in vivo* (carrageenan-induced rat paw edema assay). The chemopreventive properties of these compounds are being carried out by measuring DNMT-1 enzymatic inhibition.

17. Assay of Metformin in Human Plasma and Urine Using Small Sample Volume and Conventional Octadecyl Silane Column; Utility in Determination of Metformin Pharmacokinetic Parameters

Raniah Gabr, Raj Padwal*, Dion Brocks. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta; *Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB

Purpose: 1-To develop a selective and sensitive high-performance liquid chromatographic method to determine metformin in human plasma and urine, using conventional reverse phase column and low specimen volume 2- To apply the assay to a pharmacokinetics of metformin in obese subjects.
Methods: Extraction of metformin and ranitidine (internal standard) from plasma and urine samples (100 µL) was performed with a 1-butanol-hexane (50:50, v/v) mixture under alkaline conditions followed by back-extraction into diluted acetic acid. Separation was carried out using a C18 column (250 mm×4.6 mm, 5 µm). A mobile phase consisting of acetonitrile: 25 mM phosphate solution (34:66, v/v) and sodium dodecyl sulfate (3 mM) was pumped at 0.7 mL/min. Two obese volunteers were administered 1000 mg metformin HCl orally and the

assay was used to measure metformin plasma and urine concentrations.

Results: Calibration curves were linear (>0.995) in the concentration ranges of 10–5000 ng/mL and 2–2000 μg/mL for metformin HCl equivalents in plasma and urine respectively. The mean absolute recoveries for 100 and 1000 ng/mL metformin HCl in plasma were 93.7 and 88.5%, respectively. The intra- and inter-day coefficients of variation in plasma and urine were <20% at the lowest, and < 7% at other concentrations. The percent error values were less than 2% in plasma while it reached ~9% in urine. The validated lower limits of quantification for metformin were 7.8 ng/mL and 1.6 μg/mL, respectively, in plasma and urine. Plasma concentrations could be followed for up to 24 h following administration of the metformin dose where the observed mean Cmax, tmax, AUC and percentage of dose recovered from 24 h urine collection were 2.42 mg/mL, 1.85 h, 11.3 mg·h/L and 40.4% respectively.

Conclusion: The method was highly sensitive and selective for metformin in human plasma and urine based on a 0.1 ml sample size. The method was also shown to be applicable for assaying metformin in human plasma and urine specimens as part of a pharmacokinetic experiment.

18. Single Stage and Tandem Mass Spectrometric Analysis of Novel Antineoplastic Curcumin Analogs

Lindsey Usher[1], Alejandro Cohen[2], Umashankar Das[1], Devanand Pinto[2], Jonathan Dimmock[1], Anas El-Aneed[1]*. [1]Drug Design and Discovery Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK; [2] Institute for Marine Biosciences (NRC-IMB), National Research Council of Canada, Government of Canada, Halifax, NS

Purpose: Curcumin, a component of the spice tumeric, is a natural product being investigated for its antineoplastic properties. However, problems with poor absorption and extensive metabolism limit the usefulness of curcumin as a therapeutic agent. Therefore, curcumin analogs have been synthesized in an attempt to overcome these barriers. This research is focused on the unusual behavior of novel curcumin-like compounds, bearing the 3,5-bis(benzylidene)-4-piperidone functional group, when they are analyzed using different mass spectrometric methods.

Methods: Ten novel curcumin analogs (belonging to three different structural families) have been analyzed using electrospray ionization- (ESI) and matrix assisted laser desorption ionization-(MALDI) tandem mass spectrometry (MS/MS). MALDI-MS analysis was performed using MALDI-ICR (Ion Cyclotrone Resonance)-MS/MS instrument with 2,5-dihydroxybenzoic acid (DHB) as MALDI matrix. ESI-MS/MS analysis was conducted using API QSTAR XL qQToF MS/MS system.

Results: Theoretically, the single stage MS and the tandem mass spectrometric (MS/MS) behavior should be very similar whether using ESI or MALDI. However, tested curcumin analogs were ionized as $[M+H]^+$ using ESI while an unexpected $[M-H]^+$ species were observed during single stage MALDI-MS. In addition, MS/MS analysis revealed substantial differences in the fragmentation patterns between ESI-MS/MS and MALDI-MS/MS. Unique fragment ions for each ionization method were identified.

Conclusion: Both MS and MS/MS dissociation behavior of novel curcumin analogs was different using MALDI-MS/MS and ESI-MS/MS. These variations are attributed to the production of $[M-H]^+$ ions during MALDI-MS/MS analysis, rather than the expected $[M+H]^+$ ions. Understanding these differences will enable us to develop specific and sensitive MS-based protocols for qualitative and quantitative compound analysis within various biological matrices.

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19. Design of New Antibiotics by Attenuating Cell Wall Biosynthesis through Inhibition of the L-rhamnose Biosynthetic Pathway in *Streptococcus Pneumonia*

Jonathan Melong[1], David Jakeman*[1,2], Stephen Beaton[2]. [1]College of Pharmacy, Dalhousie University; [2]Department of Chemistry, Dalhousie University

Introduction: Bacterial resistance is emerging as an increasing public health concern creating the need to investigate new antibacterial targets to design novel antibiotics. L-Rhamnose is a sugar that plays an important role in bacterial virulence factors and

cell wall synthesis. Its biosynthesis is highly conserved among bacterial and is absent in mammalian species, making it a prime target for antibacterial design. Cps2L is an enzyme involved in the L-rhamnose biosynthetic pathway. Developing inhibitors and substrate derivatives of Cps2L may inhibit the biosynthesis of L-rhamnose; resulting in decreased bacterial virulence. The objective of this project is to identify potential substrates and inhibitors of Cps2L to guide the design of novel antibiotics against Gram-negative and Gram-positive bacteria.

Methods: Recombinant Cps2L was used in invitro assays to determine substrate binding and inhibition. Product conversion was analyzed by subjecting the supernatant to HPLC analysis at a wavelength of 254 nm. Activity was determined through the calculation of relative percent product conversion.

Results: Inhibition of Cps2L was monitored in the forward and reverse direction in the presence of various bisphosphonates. IC_{50} values calculated for methylene bisphosphonate, monofluoromethylene bisphosphonate and difluoromethylene bisphosphonate were 2.70 mM, 3.63 mM, and 1.38 mM, respectively. Forward and reverse inhibition of Cps2L was also noted in the presence of commercially available and clinically used bisphosphonates. IC_{50} values calculated for clodronate, pamidronate, and ibandronate were 4.70 mM, 10.13 mM, and 10.24 mM, respectively. Associated K_i values were also calculated.

Conclusions: Inhibition of Cps2L by various bisphosphonates has been demonstrated. Although future work needs to be completed, structurally modified bisphosphonates may serve as novel antibiotics against Gram negative and Gram positive bacteria.

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20. Design, Synthesis and Evaluation of Antimicrobial Peptide Leucocin A by Native Chemical Ligation

Krishna Chaitanya Bodapati, Rania Soudy, and Kamaljit Kaur. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

Introduction: Bacteriocins are ribosomally synthesized antimicrobial peptides derived from lactic acid bacteria to destroy competing microorganisms. Class IIa bacteriocins are useful for food preservation because of their activity against a variety of important food pathogens, including *Listeria monocytogenes* in nanomolar concentration. Leucocin A is a 37 amino acid (aa) long, cationic class IIa bacteriocin which has a characteristic, conserved YGNGVXC sequence and a disulphide bond near N-terminal region. The N-terminal portion is highly conserved and the C-terminal region has variable sequences. The C-terminal, however, folds into a conserved amphipathic α-helical structure.

Purpose: The aim of the present study was to synthesize Leucocin A by using Native Chemical Ligation (NCL) with the idea of obtaining better purity and higher yields when compared to conventional solid phase peptide synthesis.

Methods: Using Fmoc SPPS, we report the chemical synthesis of Leucocin A by conventional stepwise solid phase peptide synthesis (SPPS) and, alternatively, by native chemical ligation. For synthesis using NCL, the peptide was divided into two segments, 1 (13aa) and 2 (24aa), the latter containing cysteine at N-terminal. Segment-1 was synthesized as C-terminal thioester using sulfonamide safety catch linker resin. The thioester peptide was subsequently ligated with segment-2 to give the full length peptide. Disulphide bond was introduced by air oxidation for 48hrs in buffer pH 8.2. Peptides were purified and characterized by using RP-HPLC and MALDI-TOF mass spectrometry. Secondary structure was elucidated using circular dichroism spectroscopy. Antibacterial activity and MIC of peptides against *Listeria monocytogenes* and *Listeria innocua* were evaluated.

Results: Active Leucocin A was successfully synthesized. NCL gave higher yields (48%) and higher degree of purity, when compared to conventional method (6%). In addition, Minimum inhibitory concentrations (MIC) values for Leucocin A synthesized by NCL was lower than that of conventional SPPS, indicating low level of racemization.

Conclusion: In the present investigation, an alternative method for the synthesis of Leucocin A in high yields and purity was reported. The MIC values of synthetic Leucocin A against *Listeria monocytogenes* and *Listeria innocua* are comparable

to that of wild type Leucocin A.

Clinical Sciences & Pharmacy Practice

21. Pharmacists' Integration of Practice Care Tools for Assessment and Documentation into Routine Patient Care

Anita M Cumbleton, University of Alberta; Lisa Guirguis, University of Alberta; Shao Lee, Alberta College of Pharmacists, Edmonton, Alberta, Canada

Purpose: The objective of this study was to explore pharmacists' integration and attitudes toward patient care tools for assessment and documentation in community pharmacy practice. Secondary objectives are to identify barriers and facilitators to the use of patient care tools.
Methods: At an interactive workshop at the 2010 Alberta College of Pharmacists meeting, pharmacists observed role models and practiced using two patient care tools for assessment and one for documentation. Pharmacists who consented and had an active patient practice were telephoned two months after the workshop. The interviews consisted of a semi-structured interview on tool use and a quantitative survey on tool use and related attitudes. The interviews were audio-recorded, transcribed verbatim, and qualitatively analyzed. Descriptive statistics were used to characterize survey data.
Results: A total of 39 pharmacists with a patient care practice consented and 22 interviews were conducted. Few pharmacists were 'almost always' to 'always' assessing (32%) and documenting (10%) patient care in the past 2 weeks. Still, pharmacists 'agreed' to 'strongly agreed' that these activities are important and were 'quite sure' they could perform them. Lack of required technology, routines, patient expectations, reimbursement, and lack of time were identified as barriers to implementing patient care tools. Strategies to help reinforce tool use included practicing new habits, using technology, starting small, using physical reminders, and recognizing benefits to practice.
Conclusion: While pharmacists had positive attitudes toward assessment and documentation, they were not routinely using the patient care tools. In the future, we hope to enhance pharmacists' integration of patient care tools by recognizing their benefits and addressing barriers.
Acknowledgement: Anita Cumbleton is an awardee of the National Summer Student Research Program Awards to Present Research Findings sponsored by GlaxoSmithKline Inc.

22. Determining Patient Attitudes Regarding the Role of the Pharmacist

Deidre Clark, Stephanie Young, Leslie Phillips, and Deborah Kelly. School of Pharmacy, Memorial University of Newfoundland, St. John's, NL

Introduction: Pharmacists are consistently ranked as one of the most trusted professionals and studies have suggested a high level of satisfaction with services provided by community pharmacists. Yet there is little research regarding patient perception of the community pharmacist's role. This study will add a Canadian perspective to the current body of knowledge on this subject.
Methods: A telephone survey of adults across Newfoundland and Labrador was conducted. A questionnaire was developed based upon literature review of relevant studies. Survey questions assessed usage of community pharmacies, knowledge of pharmacist functions, general opinions about pharmacists, and likelihood of availing of expanded pharmacist services. Residential phone numbers were called (60% urban/40% rural). Surveys were conducted by undergraduate students and the data was entered into a MS Access database. Descriptive statistics were performed using IBM SPSS software (v.19).
Results and Discussion: A total of 380 surveys were completed. Detailed results will be presented. Results indicate general public awareness of the tasks involved in filling a prescription, but not of some of the higher cognitive functions a pharmacist performs. People are comfortable sharing their health information with the pharmacist, and want pharmacists and physicians to work together for optimal care. The majority of respondents showed interest in private medication consultations with the pharmacist; fewer were interested in receiving vaccinations or health screening tests.
Conclusion: The results support the view that pharmacists are trusted health professionals. People are aware of most of what pharmacists do, but an opportunity exists to better educate the public on our knowledge and unique professional abilities, to provide enhanced patient care, and to expand

pharmacy services.

Acknowledgement: Deidre Clark is an awardee of the National Summer Student Research Program Awards to Present Research Findings sponsored by GlaxoSmithKline Inc.

23. PEG-Caffeine Conjugate as a Polymeric Binder for Heavy Metal Detoxification

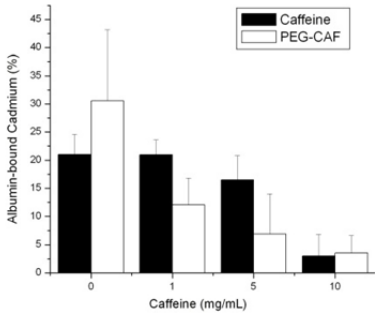
Jeanne Leblond[†], Nicolas Bertrand[†], Céline Bouvet[†], Anne Petitjean[‡], Pierre Moreau[†] and Jean-Christophe Leroux[§]. Contribution from [†] Faculty of Pharmacy, University of Montréal, Montreal, QC, Canada ; [‡]Department of Chemistry, Queen's University, Kingston, ON, Canada and [§] Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, ETH Zürich, Zürich, Switzerland.

Purpose: Polymeric binders are able to sequester endogenous or exogenous substrates, and subsequently modify their physicochemical/biological properties. In this work, we report a polymeric binder for *in viv*o biodetoxification of heavy metals, of which cadmium (Cd^{2+}) was selected as an illustrative model. The strategy explored herein relies on the use of a functionalized 8-arm branched PEG which could bind cadmium in the blood circulation, decrease its tropism toward the liver, and ensure its renal elimination.

Methods: The polymeric binder was designed from an 8-arm PEG. The molecular weight (Mw = 20 000) was chosen to achieve a long circulation time (to maximize the chances of cadmium capture) while still allowing renal excretion. The binding affinity was provided by terminal functionalization with caffeine units, which have been reported to bind cadmium through cationic–aromatic interactions. Hence, the multibranched PEG was coupled to theophylline leading to a caffeine-like conjugate (PEG-CAF). The grafting efficiency was measured by ^{1}H NMR, spectrophotometry and elemental analysis. The binding affinity for zinc and cadmium was determined by FTIR and UV spectroscopic assays. Competition assays in the presence of albumin were conducted using a HiTrap Blue affinity column for albumin and Cd^{2+} was measured by flame atomic absorption. Pharmacokinetics, biodistribution and hemodynamic parameters were determined in Sprague-Dawley male rats over a period of 24h.

Results: The multi-branched PEG–caffeine conjugate was easily achieved by a two-step procedure (95% grafting for a yield of 55%). It was confirmed that the caffeine moieties associate Cd^{2+} even conjugated to PEG (3.74×10^5 M–1). *In vitro*, PEG-CAF was able to displace cadmium bound to albumin in a dose-dependent manner. *In vivo*, PEG-CAF was characterized with a long circulation time after *i.v.* injection ($t_{½}$ = 14 h), with limited accumulation in the liver after 24 h. The conjugation of caffeine to the branched PEG abolished its hemodynamic effects, as shown by the stability of hemodynamic parameters after *i.v.* administration of PEG-CAF.

Conclusion: These preliminary experiments demonstrate the substantial promise of PEG-CAF as a detoxifying agent.



24. Prevalence, Awareness, Treatment and Control of Hypertension and Associated Risk Factors within the PURE-Quebec Cohort

Catherine Julien[1,2], Ginette Turbide[2], Stéphanie Lacroix[3], Samantha Kellett[3], Gilles R. Dagenais[2,3], Paul Poirier[1,2]. 1. Faculty of Pharmacy, Laval University, Quebec, Canada; 2. Institut universitaire de cardiologie et de pneumologie de Québec, Quebec, Canada; 3. Faculty of Medicine, Laval University, Quebec, Canada

Purpose: Hypertension is an important risk factor for cardiovascular diseases (CVD). The PURE (Prospective Urban and Rural Epidemiology) study is a worldwide prospective observational study aiming to assess the impact of environmental and social factors on heart diseases, diabetes and obesity. The main goals of this project were to 1) Estimate the prevalence of hypertension known and unknown, its treatment and its control within the PURE-Quebec sample and, 2) assess the impact of associated factors (age, sex, body mass index [BMI],

LDL, Apo B, history of CVD or diabetes, educational level, familial income and structural environment [urban vs rural]) on the prevalence, awareness, treatment and control of hypertension.
Methods: The 2789 participants from de PURE-Quebec cohort (aged between 35 and 70 years old) were met for a baseline visit between 2006 and 2009 to collect information regarding hypertension and associated factors, among others. Two blood pressure measurements were taken with the OMRON-757 electronic device. Prevalence of hypertension, awareness, treatment and control were obtained, first for the whole cohort, then for the different categories of associated factors.
Results: A global prevalence of hypertension of 37,2% was observed. Within participants with hypertension, 50,5% were aware of their condition. In those aware, 91,8% were under antihypertensive medication. 50,0% had their hypertension controlled with the medication. All the associated factors analyzed (except LDL level) have an impact on the prevalence of hypertension ($p<0,05$). Concerning the awareness of hypertension: age, sex, BMI, smoking status, LDL, Apo B and history of CVD or diabetes seems to be playing a role ($p<0,05$). For the treatment proportion, only age seems to have an influence ($p<0,05$). For the control of hypertension, sex, BMI, history of diabetes and educational level appears to have an effect ($p<0,05$).
Conclusion: The prevalence of hypertension is high in the PURE-Quebec Cohort. The awareness level and the control level are quite low. Many demographic (age, sex, structural environment), metabolic (BMI, Apo B, LDL), pathological (CVD, diabetes) and socioeconomic factors (educational level and familial income) are associated with the prevalence, awareness, treatment and control of hypertension.
Acknowledgement: Catherine Julien is an awardee of the National Summer Student Research Program Awards to Present Research Findings sponsored by GlaxoSmithKline Inc.

25. Humoral and Cellular Immune Responses of Quality Controlled Hepatitis B Plasmid DNA Vaccine

Mounir Salem-Bekhit[1], Ibrahim El-Bagory[1], Mohsen Bayomi[1], Mohammed Gad-El-Rab[2] and Mohammed Fathallah[3]. [1]Department of Pharmaceutics, College of Pharmacy; [2]College of Medicine, King Saud University, Riyadh Saudi Arabia; [3]Biotechnology Program, College of Graduate Studies, King Fahd Chair for Health Biotechnology, Arabian Gulf University, Manama, Bahrain.

Purpose: To control the quality and integrity of a hepatitis B plasmid DNA vaccine as active pharmaceutical ingredient (API) and detect the immunization responses following intramuscular injection of the plasmid in mice.
Methods: A naked plasmid DNA (gWizHBs) encoding the hepatitis B virus S antigen was propagated in *Escherichia coli* DH5α and analyzed by agarose gel electrophoresis. DNA restriction profiling and sequencing were carried out. The characterized plasmid DNA was used to vaccinate Balb/c mice by intramuscular route. The animals were injected with different doses of the characterized plasmid DNA and boosted by 10 µg two weeks later. The humoral immune response was monitored by ELISA while the cellular immune response was investigated through analysis of the spleen cytokine profile [TNFα, IFN γ, and IL2] as well as the CD69 expression level in CD4 and CD8 positive cells.
Results: Around 90% of the produced plasmid DNA was of high quality (supercoiled DNA). The DNA sequencing showed full integrity of plasmid elements. The production of pharmaceutical grade hepatitis B plasmid DNA vaccine as API was utilized using established master cell bank. In immunization study, the serum antibody showed first an IgM response at weeks 2-3 followed by IgG response that appeared at week 3 and lasted for 6 weeks. This serum antibody level varied with the concentration of plasmid DNA injected and the highest level was obtained when the animals was immunized using 10 µg of gWizHBs. The cytokine profile showed also high levels of TNFα, IFN γ, and IL2 and CD69 expression in the group of animal immunized using 10 µg dose.
Conclusion: Quality control was assured for the production of high quality and maximum amount of hepatitis B plasmid DNA vaccine. Intramuscular injection of the modified vaccine of 10µg dose in mice induced high humoral and cellular responses.
Acknowledgement: Project was supported by Center of Excellence in Biotechnology Research, King Saud University, Riyadh, Saudi Arabia.

Pharmacokinetics & Pharmacodynamics

26. The Investigation of Variability of the Pharmacokinetics of Mesalamine from Various Mesalamine Formulations in Randomized Clinical Trials

Juan He, Masood Bhatti, Lorelei Lutter. BioPharma Services Inc. Toronto, ON, Canada

Purpose: Mesalamine, which is also called 5-aminosalicylic acid or 5-ASA, is an amino-salicylate anti-inflammatory drug indicated for the treatment of ulcerative colitis. There are several oral delayed release and sustained release mesalazine formulations. The pharmacokinetic profiles of Asacol®, Pentasa®, and Lialda® and generic counterparts are included in this investigation.
Methods: Six comparative bioavailability trials in healthy volunteers with different mesalamine formulations were selected. Five studies were randomized two-way crossover with two periods, two sequences in healthy volunteers, and one study was the three-way crossover with two study products and one product given twice. The sample sizes employed in those clinical studies were from 12 to 50. The quantification of pharmacokinetic data included at a minimum, 5-ASA from all 6 trials, and its major metabolite N-Acetyl-5ASA from 2 trials. Pharmacokinetic data (Tmax, Cmax, AUC, and ka) of 5-ASA, and its major metabolite N-Acetyl-5ASA (if available) were calculated. The pharmacokinetic analyses were carried out by either WinNonlin or SAS.
Results: Different oral mesalamine dosage forms result in widely different mesalamine plasma profiles. The median Tmax of mesalamine was around 10 to 12 hours for Asacol® and Lialda®; while the maximum plasma concentration of mesalamine was reached around 2 to 3 hours after the administration of Pentasa®. The summary results for peak and systemic absorption of 5-ASA from all three formulations showed large variability for the parent compound 5-ASA. The intra-subject variability for both Cmax and AUC was above 85% from the delayed release formulations of both Asacol® and Lialda®; the intra-subject variability for both Cmax and AUC was above 50% and 38% respectively for extended release formulation Pentasa®.
Conclusion: The systemic and peak exposure to 5-ASA, which are main parameters of the comparative bioavailability assessment, showed large variability for all 6 trials. The delayed release products showed higher variability than the extended release product. Such variability introduces the complexity for the planning of the comparative bioavailability study in terms of the determination of the sample size. Caution needs to be taken.

27. Bioequivalence Study of Glucosamine Hydrochloride and Glucosamine Sulfate in the Rat and Human

Ali Agahzadeh-Habashi and Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Purpose: The effectiveness of glucosamine (GlcN) in the treatment of osteoarthritis is inconclusive. Most positive results are generated following administration of a crystalline GlcN sulfate (GlcN-S) that is marketed by Rottapharm (Manza, Italy). In addition, this product seems to demonstrate higher plasma concentrations than GlcN HCl. Data directly comparing GlcN-S and the HCl salts are not available. We examined bioequivalence of GlcN HCl and GlcN-S following administration to humans and rats.
Methods: Five male Sprague-Dawley rats were used in animal study and four healthy individuals took part in human study. GlcN HCl or GlcN-S was administered to animals at a dose of 100 mg/kg (normalized for GlcN HCl) using oral gavages in an open-label, randomized, cross-over fashion. Plasma samples were collected before dosing and at given times post-dosing for 4 h. Human volunteers received one single dose of either salts at 1500 mg (normalized for GlcN HCl) as a single-dose in an open-label, cross-over fashion. Urine samples were collected in given times for 13 h. Washout period for animal and human studies were 2 and 14 days, respectively. Plasma and urine sampled were analyzed for glucosamine using validated high performance liquid chromatography with fluorescence detector. The pharmacokinetic parameters were determined and for bioequivalence determination the difference of C_{max}, AUC_{0-t} and Xu_t were analyzed by ANOVA and 90% confidence interval.
Results: The mean (SD) of pharmacokinetic parameters of GlcN HCl and GlcN-S were as below.

Parameters	GlcN HCl	GlcN-S
$t_{1/2}$ (h) $^{\alpha}$	0.67	0.85
t_{max} (h) *	1.50 (0.73)	1.30 (0.33)
C_{max} ($\mu g.L^{-1}$) *	7.49 (2.76)	7.92 (1.84)
AUC_t ($\mu g.h.L^{-1}$) *	13.59(3.64)	12.50 (2.55)
Xu_{13} (mg) ‡	37.95 (2.12)	29.15 (7.23)

$^{\alpha}$ calculated by using averaged plasma concentrations. *Animal data, ‡ Human data

The 90% confidence interval of mean difference of C_{max}, AUC_{0-t} and Xu_{13} (log-transformed data) of GlcN HCl compared with GlcN-S formulations were 94.93 %(88.69-101.17%) and 102.27% (89.71-114.83%) and 108.59% (103.07-114.11%), respectively.

Conclusion: Glucosamine hydrochloride and crystalline glucosamine sulfate are bioequivalent and based on the result of this small animal and human study. There is no superiority of one glucosamine salt over the other at least in terms of pharmacokinetics and oral bioavailability.

28. Effects of Inflammation on the Action and Disposition of Nebivolol

Forugh Sanaee and Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Inflammation reduces clearance of drugs with efficient hepatic metabolism. In addition, pharmacological response to beta-adrenergic blockers is reduced by inflammation even in the presence of high concentrations. The aim of this study was to investigate the effect of inflammation on the action and disposition of nebivolol, a third generation beta-blocker with NO-generating property that undergoes first pass metabolism.

Method: We studied pharmacokinetics and pharmacodynamic of nebivolol in two groups of Male Sprague-Dawley rats, Control and Inflamed. On day 0, rats received either single injections of 0.2 mL Mycobacterium butyricum in squalene (Inflamed) or the same volume of 0.9% normal saline (Control). On day 15, when adjuvant arthritis (AA) was confirmed, rats were anesthetized and ECG electrodes were implanted. On day 16, the baseline (pre-drug dosing) PR and RR interval were recorded. Single oral racemic nebivolol doses of 2 mg/kg were administered through a gastric gavage and ECG recorded at 15, 30, 45, 60, 90, 120, 180, 210, 240, 270, 300, 330 and 360 min. Subsequently, the right jugular veins were cannulated. On day 17 and after a 12 h fasting, the rats received 2 mg/kg nebivolol. Serial blood samples (200 µL) were collected at 0, 15, 30, 45 min, 1, 2, 3, 4, 6, 8, 12 and 24 h post nebivolol dosing.

Results: Both groups of animals demonstrated prolongation of PR interval in response to nebivolol with no significant inter-group differences (change from baseline Control vs AA: Maximum effect, 22.9%± 12.6 vs 27.4%±6.2; area under the effect-time curve, 2670±3222 vs 5300±2158 (% × min). Neither did AA influence plasma nebivolol concentration (AUC, Control, 717±296: AA, 577±221 ng×h/mL).

Conclusion: Surprisingly, the experimental arthritis did not reduce clearance of nebivolol, a drug with extensive first-pass effect. Similarly, the pharmacological response to nebivolol remained unchanged in the presence of inflammation, an observation different from those for other investigated beta-blockers. This lack of effect of AA on response to nebivolol may be due to the drug's nitric oxide generating property. The lack of effect of on nebivolol pharmacokinetics may indicate a clearance pathway different from other efficiently cleared drugs.

29. Pharmacokinetics of Nebivolol in the Rat: Low Oral Bioavailability due to Loss in the Gut

Forugh Sanaee and Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Science, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Nebivolol is a third generation β-blocker with vasodilatory properties through the NO mechanism with a reported bioavailability of 12% following oral doses and is thought to be exclusively metabolized in the liver. The aim of this study was to evaluate the pharmacokinetics of nebivolol following various routes of administration and to investigate whether intestinal metabolism also contributes to the first-pass metabolism of nebivolol in the rat.

Method: Male Sprague-Dawley rats (400 ± 10g) were cannulated in the right jugular veins. Single 1 mg/kg i.v. or i.p. (n=8/group) and 2 mg/kg oral (n=10, via gastric gavage) doses were administered.

Following i.v. injection, the cannula was washed with normal saline before the first blood sample collection. Serial blood samples (200 μL) were collected prior and up to 24 h post drug dose and plasma nebivolol concentrations were determined using HPLC. The systemic availabilities of i.p and p.o. nebivolol doses were determined as compared with the i.v. doses by dividing the area under the plasma concentration-time curves (AUC_{0-24}) of the former routes by the latter.

Results: There was no significant difference between AUC values following i.v. and i.p. doses while the oral dose produced significantly lower bioavailability.

	Tmax, h	Cmax, ng/mL	AUC, ng.h/mL	F
		Corrected for 1 mg/kg dose		
i.v.	nd	nd	869±456	1.00
i.p.	3.2±2.7	239±257	726±675	0.84±0.78
p.o.	3.2±2.1	72.2±35.3*	330±132*	0.38±0.15*

nd, not determined; *, significant difference from other routes.

There was no significant difference between the t1/2 values following different routes (4.2±2.8 h for i.v., 7.0±4.3 h for i.p. and 4.0±3.2 h for oral).

Conclusion: Oral doses of nebivolol demonstrate low bioavailability. Since i.p. doses have equal AUC values to i.v. doses, the loss of bioavailability after oral doses is likely due to either lack of oral absorption or metabolism in the gut.

30. Effect of Isoproterenol on Cardiovascular Hemodynamics, RBC Concentrations of Adenine Nucleotides, and Mortality in a Freely Moving Rat Model *in vivo*

Pollen K.F. Yeung and Dena Seto. Pharmacokinetics and Metabolism Laboratory, College of Pharmacy and Department of Medicine, Dalhousie University, Halifax, NS, Canada

Purpose: To study the effect of isoproterenol on cardiovascular hemodynamics, RBC concentrations of adenine nucleotides and mortality in a freely moving rat model *in vivo*.

Methods: Sprague Dawley (SD) rats with a carotid artery catheter weighing between 250 and 300g were used. Each rat was acclimatized to the Carleton Animal Care Centre and free access to food and water for at 48 hours before experiment. Each rat was housed in a freely moving caging environment with free access to drinking water. In the treatment group (n = 10), isoproterenol (30 mg/kg) was administered by subcutaneous injection 1 hour after the animal was settling in the cage. A separate group without receiving isoproterenol was used as control (n = 9). Blood samples were collected at 0, 0.05, 0.25, 1, 1.2, 1.5, 2, 3, 4, 5 and 6 hours for measurement of adenine nucleotides (ATP, ADP and AMP) by a validated HPLC. Hemodynamic recording (SBP, DBP, and HR) was collected throughout the experiment using a TruWave® disposable pressure transducer (Model PX601, Edwards Lifesciences Canada, Inc., Mississauga, ON, Canada) coupled to a Siemens hemodynamic monitor (Sirecust 400) and chart recorder (Siredoc) (Erlangen, FRG). RBC concentrations of Data were analysed using *t*-tests and difference considered significant at $p < 0.05$.

Results: Isoproterenol induced 50% mortality within 4 hours after administration ($p < 0.05$). It decreased SBP and DBP immediately after the injection (< 15 min) by -64 ± 22 and -64 ± 20 mmHg, but increased HR by +158 ± 59 bpm at the end of the experiment ($p < 0.05$). Both SBP and DBP rebounded to pre-treatment (baseline) level after 1-2 hours after injection ($p < 0.05$), and then continued to fall for the remaining of the experiment. There was no rebound from the HR response. Isoproterenol also increased RBC concentrations of AMP from 0.04 ± 0.01 to 0.28 ± 0.23 mM (+500%) at the end of the experiment ($p < 0.05$). The rats that died had greater increase of the AMP concentration than those surviving one.

Conclusion: Isoproterenol traumatically decreased SBP and DBP, but increased HR. It also increased RBC concentrations of AMP particularly in the dying rats (Supported in part by CIHR, Nova Scotia Health Research Foundation and Dalhousie Pharmacy Endowment Foundation).

31. Exercise Pre-conditioning Attenuates Cardiovascular Toxicities and Reduces Mortality Induced by Isoproterenol in a Freely Moving Rat Model *in vivo*

Pollen K.F. Yeung and Dena Seto. Pharmacokinetics and Metabolism Laboratory, College of Pharmacy and Department of Medicine, Dalhousie University, Halifax, NS, Canada

Purpose: To study the effect of exercise pre-conditioning on cardiovascular toxicities and

mortality induced by isoproterenol in a freely moving rat model *in vivo*.

Methods: Sprague Dawley (SD) rats with a carotid artery catheter weighing between 250 and 300g were used. Each rat was acclimatized to the Carleton Animal Care Centre (CACC) and had free access to food and water for 48 hours before experiment. Each rat was housed in a freely moving caging environment during experiment with free access to drinking water. In the group with exercise pre-condidtioning (n = 8), each rat was exercised on a treadmill for 15 minutes at 14 m/min 2 hours before receiving isoproterenol (30 mg/kg) by subcutaneous injection. Two separate groups with one without receiving isoproterenol and exercise (n = 9); and the other without exercise (n = 10) were used as control. Blood samples were collected at 0, 0.05, 0.25, 1, 1.2, 1.5, 2, 3, 4, 5 and 6 hours for measurement of adenine nucleotides (ATP, ADP and AMP) by a validated HPLC. Hemodynamic recording (SBP, DBP, and HR) was collected for the duration of the experiment using a TruWave® disposable pressure transducer (Model PX601, Edwards Lifesciences Canada, Inc., Mississauga, ON, Canada) coupled to a Siemens hemodynamic monitor (Sirecust 400) and chart recorder (Siredoc) (Erlangen, FRG). Biomarker data were analysed using *t*-tests and difference considered significant at $p < 0.05$.

Results: Exercise pre-conditioning reduced mortality induced by isoproterenol within 4 hours after injection from 50% to 20%. It reduced the abrupt rebound of mean SBP from +75 ± 41 to +41 ± 15 mmHg (t-test, $p < 0.05$) and DBP from + 76 ± 37 to +39 ± 25 mmHg ($p < 0.05$), but had no effect on the increase of HR. It also attenuated the increase of RBC AMP concentrations from +0.24 ± 0.23 mM in non-exercise rat to + 0.07 ± 0.11 mM in rats after the exercise pre-conditioning (t-test, $p = 0.074$).

Conclusion: Exercise pre-conditioning reduced cardiovascular toxicities, RBC concentrations of AMP and mortality induced by isoproterenol (Supported in part by CIHR, Nova Scotia Health Research Foundation and Dalhousie Pharmacy Endowment Foundation).

32. Optimizing Drug Development of TP-434, a Novel Fluorocycline, with Adaptive Learn and Confirm Cycles of Modeling and Simulation

Corinne Seng Yue[1,2], Joyce A Sutcliffe[3], Philippe Colucci[1,2], Craig R Sprenger[2], Murray P Ducharme[1,2]. [1]Université de Montréal, Faculté de pharmacie, Montréal, QC, Canada, [2]Cetero Research, Cary, NC, USA, [3]Tetraphase Pharmaceuticals, Watertown, MA, USA

Purpose: This analysis aimed to determine which dosing regimens should be evaluated in a multiple ascending dose (MAD) study of TP-434, a novel fluorocycline that is being developed.

Methods: A first-in-man, single ascending dose (SAD) study with TP-434 was conducted. TP-434 was given as a 30-minute IV infusion at doses of 0.1, 0.25, 0.5, 1, 1.5, 2 & 3 mg/kg (6 subjects received TP-434 & 2 subjects received placebo in each cohort). Population pharmacokinetic (PK) analyses were done with ADAPT 5 after the completion of each SAD cohort using plasma & urinary data. The best model was chosen using standard model discrimination criteria. Simulations were then performed with the model to obtain clinical endpoints associated with various dosing regimens, including AUC/MIC (area under the concentration-time curve (AUC)/ minimal inhibitory concentration (MIC)), T>MIC (% time drug concentration exceeds MIC at steady-state) and Cmax/MIC. MAD regimens were proposed using these endpoints.

Results: Seven cycles of modeling & simulation were conducted with data from 42 subjects. TP-434 PK was described by a 4-compartment model with linear elimination. Mean parameters (% intersubject variability) were Vc = 10.8 L (20.6%), CLnr = 11.5 L/h (19.5%), Vp1 = 16.1 L (23.8%), CLd1 = 44.3 L/h (8.69%), Vp2 = 132 L (20.2%), CLd2 = 6.95 L/h (40.8%), Vp3 = 103 L (25.1%), CLd3 = 26.9 L/h (30.8%) & CLr = 2.34 L/h (18.4%). Residual variability for plasma & urine data was 12.7% & 21.9%, respectively. When only AUC/MIC was considered, model-based simulations suggested that a minimum of 1.5 mg/kg QD for 10 days would be efficacious for organisms with an $MIC_{90} = 2$ mg/mL.

Conclusion: The PK of TP-434 was well-described by a 4-compartment model. Using model-based simulations, the dosing regimens originally proposed for the MAD study were modified prior to study initiation to evaluate more appropriate regimens.

Please note that the results presented in this abstract were presented as a poster at the 50th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, which was held from September 12-15, 2010 in Boston, MA. The poster number was A1-028 and it was presented on September 12, 2010.

Drug Delivery & Pharmaceutical Technology

33. New Immediate Release Formulation for Deterring Abuse of Methadone

A.O. Betancourt[1], P. Gosselin[1*] and R. Vinson[2]. [1]Corealis Pharma Inc, Laval, Qc, Canada; [2]Paladin Labs Inc, St-Laurent, Qc, Canada.
[*]Corresponding author

Purpose: The present paper describes a new abuse-deterrent formulation which is suitable for many active pharmaceutical ingredients, but is most relevant to narcotic drugs, including the opioids oxycodone, morphine, hydromorphone, hydrocodone, methadone and other acidic drugs with a chemical structure containing at least one positively-charged protonated amine group. The addition of an alkalizing agent causes the active pharmaceutical ingredient to precipitate out of an aqueous solution along with other ingredients and is retained on standard filters used to prepare a solution for illicit drug use, for instance intravenous injection.
Methods: In this study, HPLC assay was used to determine how alkalizing agents prevented extraction of the opioid component of tablets in aqueous and alcohol solvents. *In vitro* dissolution testing was used to determine drug release from tablets in different media. Tablets combining methadone as an opioid and meglumine as alkalizing agent were manufactured by dry blending and direct compression and evaluated for physicochemical properties.
Results: The reduction in water solubility depended on the nature of the alkalizing agent and on the methadone / alkalizing agent ratio. Meglumine-based formulations could prevent extraction of 70% to 100% of the methadone from 0.5 mg/mL aqueous solutions. Using similarity factor (f_2) as comparative criteria, meglumine-containing and control tablet formulations showed similar dissolution profiles in acidic media, suggesting adequate solubilisation of the drug early in the gastrointestinal tract.
Conclusion: Incorporation of an alkalizing agent into the methadone tablet formulations significantly reduced or completely prevented the preparation of a methadone solution for possible intravenous administration and abuse, while allowing the pharmaceutical formulation to release the active pharmaceutical ingredient in gastric media in order to deliver the desired pharmacological effect. This new formulation is the basis for a new tablet preparation of Metadol®, an immediate-release methadone product available in Canada.

34. Anti-inflammatory Potential of Artificial Microcapsules Containing Thalidomide: in-vitro Analysis

Marc Fakhoury, Michael Coussa-Charley, Arghya Paul, Laetitia Rodes, Wei Shao, Hani Al-Salami, Imen Kahouli, Satya Prakash*. Biomedical Technology and Cell Therapy Research Laboratory, Departments of Biomedical Engineering, Physiology, and Artificial Cells and Organs Research Center, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

Purpose: Crohn's disease is a chronic inflammatory bowel disease associated with an abnormal immune response in the gastrointestinal tract. Several studies demonstrate that Thalidomide could be effective in the treatment of refractory Crohn's disease. However, its widespread usage have been limited because of potential side effects. In the present study we investigated the inhibitory activity of APA microcapsules containing Thalidomide on Lipopolysaccharide (LPS)-induced inflammation in RAW 264.7 macrophages.
Methods: cell viability was assessed by MTS assay, and nitric oxide (NO) concentration in the cultured medium was determined by Nitric Oxide Colorimetric Assay Kit (Biovision). Inflammatory cytokines released from LPS-induced RAW 264.7 macrophage cells were measured in the cell culture supernatants at several time points using commercially available ELISA Kit.
Results: Treating RAW 264.7 macrophage cells with encapsulated Thalidomide altered the overall macrophage phenotype. This resulted in a significant reduction in the production of Tumor necrosis factor-α (TNF-alpha), Interleukin-1β (IL-1β) and Interleukin-6 (IL-6). Moreover, the concentration of NO in cell supernatant was suppressed by almost 50% after 48h incubation with Thalidomide.
Conclusion: These data suggest that Thalidomide plays an important role in reducing the extent of inflammation in LPS-induced RAW 264.7 macrophage cells. Moreover, the present project validates the efficiency of APA microcapsules in providing a targeted delivery of Thalidomide, which

could be useful in treating chronic conditions such as Crohn's disease.

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35. Deposition of Alginate on a Stainless Steel Substrate for Industrial Biomedical Applications

Chris Jackson, Hani Al-Salami, Raja Chemali, Satya Prakash***.** Biomedical Technology and Cell Therapy Research Laboratory, Departments of Biomedical Engineering, Physiology, and Artificial Cells and Organs Research Center, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

Purpose: The biopolymer alginate has been widely used in many pharmaceutical formulations over the last few decades. Alginate is a straight-chain polysaccharide composed of two monomers: mannuronic acid and its C-5 epimer guluronic acid. Of recently, alginate microcapsules have been used for drug delivery, cellular encapsulation, artificial cells and bioreactors. The formation of alginate gel through electrophoretic deposition has recently gained attention as the process is already used on ceramics to create biocompatible coatings of implants in a cost effective and highly customisable manner. Alginate has already been co-deposited with HA, Chitosan, carbon nanotubes, proteins and cells, but its detailed characterization has been poorly studied.

Methods: Alginate was deposited on thin rectangular stainless steel foil. The voltage was varied between 3 and 30 volts, the distance between electrodes varied between 20 and 200 mm, and alginate concentrations were varied between 0.1% and 4%. The deposition rates, hydrogel mechanical characteristics and hydrogel alginate concentration were measured.

Results: The mechanical properties and alginate concentrations of the resultant gel increased proportionally with alginate concentration. The deposition uniformity was low, with more deposit at the extremities of the substrate than at the centre. The deposition rates increased with voltage, current, concentration, and decreased with distance between electrodes.

Conclusion: The data did not follow the Hamaker equation. The deposition overvoltage varied greatly with alginate concentration, distance between electrodes, and substrate size and geometry. Thus, electrophoretically deposited alginate holds significant promise in therapeutics. The low cost of the process and high flexibility open up new possibilities in the design of low-cost biomedical devices.

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36. Evaluation of the Effect of Dispersion Media on Magnesium Hydroxide and Aluminum Hydroxide Suspensions

Prathyusha Lella[1], Yingzhe Wang[1], Kenneth S. Alexander[1]. [1]College of Pharmacy, Industrial Pharmacy Division, The University of Toledo, Toledo OH, USA.

Purpose: The objective of the study was to investigate the effect of dispersion media on the sedimentation characteristics of magnesium hydroxide and aluminum hydroxide suspensions.

Methods: Various quantities of dry powder magnesium hydroxide and aluminum hydroxide (15, 20, 25, 30 and 35 Gm) were weighed and added to 150 mL of Purified Water USP in a 250 mL graduated cylinder and wetted for 24 hours. After this time period, the volume was made up to 200 mL and shaken 20 times. The change in the height of the interface was recorded with respect to time. This process was repeated using different concentrations of CMC (0.005, 0.01 and 0.02%) as the dispersion medium for magnesium hydroxide, and PEG 1000 (0.01, 0.03 and 0.05%) for aluminum hydroxide. The effect of the polymers in the dispersion medium was evaluated using the hindered settling theory, SEM and Laser Diffraction.

Results: The rate of fall was calculated by plotting interface height versus time. The effect of dispersion media on the particle size was investigated using the Richardson & Zaki, Steinour, and Dollimore & McBride equations. The hindered settling theory showed that CMC and PEG 1000 stabilized magnesium hydroxide and aluminum hydroxide suspensions, respectively, with a higher rate of fall of particles, increasing viscosity, and a slightly enlarged particle size. The initial radius for magneisum hydroxide was 9.104 μm, and increased with increasing concentrations of CMC to a maxium

of 12.394 μm; while aluminum hydroxide went from 43.258 μm to 47.786 μm using PEG 1000. The effectiveness of hindered settling theory based on the assumptions of the shape of dispersed particle, was questioned. Magensium hydroxide particles turned out to be clusters of random shaped solids, while aluminum hydroxide particles were more spherical, and fitted the theory better. However, the calculated radii for both the dispersed solids were within the range of particle size obtained from SEM and Laser Diffraction.

Conclusions: The results indicated that the stabilizing effects increased with increasing polymer concentration; however, when the concentration increased over a certain value, the system de-flocculated. This might be attributed to the polymer nature, and interaction with the dispersed solids. The hindered settling theory is an important approach for characterization of concentrated suspensions, although the particle shape assumption affected its application to some extent.

37. Micro/nanonization of Naproxen and Beclomethasone Dipropionate in Aqueous Environment by Femtosecond Laser Fragmentation

Weimeng Ding[1], Jean-Philippe Sylvestre[1], Grégoire Leclair[2] and Michel Meunier[1]. [1]Canada Research Chair in Laser Micro/Nano-engineering of materials, Laser Processing and Plasmonics Laboratory, Department of Engineering Physics, École Polytechnique de Montréal, Montréal, Québec, Canada; [2]Faculty of Pharmacy, Université de Montréal, Montréal, Québec, Canada

Purpose: Controlling the particle size of drugs is a simple method that may be used to improve their bioavailability, especially for compounds delivered by inhalation and orally. In the case of pulmonary delivery, micronization techniques are frequently used to optimize particles deposition in the lung. In the case of oral delivery, nanonization of poorly water-soluble drug particles accelerates their dissolution and therefore improves their absorption. However, current micro/nanonization techniques are not well adapted to the drug discovery stage, where the availability of the actives is scarce (~10-100 mg). We propose a novel approach, laser fragmentation, to perform micro/nanonisation of drugs using small quantities. This study investigates the production of drug micro/nanocrystals by laser fragmentation and evaluates the effects of the process on their physicochemical properties. Two drug models are investigated: naproxen, an anti-inflammatory drug administered orally, and beclomethasone dipropionate, a pulmonary drug used for the treatment of asthma.

Methods: Laser fragmentation consists in focusing a laser radiation into a magnetically agitated drug suspension. In this study, a femtosecond laser was used. The drugs particle size was characterized by dynamic light scattering (DLS), laser diffraction (LD) and scanning electron microscopy (SEM). The degradation was evaluated by high performance liquid chromatography (HPLC). The physicochemical properties of the lyophilized micro/nanocrystals were evaluated by Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), elemental analysis (EA) and differential scanning calorimetry (DSC).

Results: Nanocrystals of naproxen and microcrystals of BDP were successfully produced by laser fragmentation. Particles of different sizes could be obtained by adjusting the fabrication parameters (laser power, suspension concentration, treatment duration, etc.) Nanonization was accompanied by more chemical degradation than micronization. After the laser process, the chemical composition was mainly conserved except for a slight increase in the OH band (by FTIR) and the oxygen content (by EA), suggesting that oxidation may occur during laser-drug interaction in water. The process had limited effect on the drugs crystallinity.

Conclusion: Laser fragmentation enables the micro/nanonization of small quantities of drugs with limited degradation and polymorphic transformation. The process therefore represents a suitable micro/nanonization technique for the drug discovery phase, and may be of particular interest for poorly water-soluble pulmonary drugs.

38. Design and Evaluation of Cyclodextrin-based Delivery Systems for Curcumin Analogs as Topical Treatment of Melanoma

Deborah Michel[1], Jackson Chitanda[2], Réka Balogh[3], Jagbir Singh[1], Umashankar Das[1], Anas El-Aneed[1], Jonathan Dimmock[1], Ronald Verrall[2], Ildiko Badea[1]. [1]Drug Design and Discovery Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan; [2]Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada;

[3]Faculty of Pharmacy, Semmelweis University, Budapest, Hungary.

Purpose: Melanoma is considered the most dangerous of the skin diseases due to its high propensity for metastasis. Our aim is to find chemical entities with high specificity towards melanoma and to combine these agents with novel delivery systems that could eliminate invasive techniques while improving the efficiency of the treatment.
Analogs of curcumin, the active ingredient of the rhizome turmeric, featuring a piperidine ring and acyclic moieties incorporated into the pharmacophore show high anticancer activity. Representatives of this family showed high in vitro cytotoxicity towards neoplasms, but low ability to increase the life span in cancer-bearing animals due to their high lipophilicity. A new class of delivery agents has been initiated by connecting cyclodextrin (CD), to cationic gemini surfactants (CDgemini). This agent was designed as an amphiphilic molecule that can incorporate the lipophilic drug into the CD pocket. The CDgemini/drug complex interacts electrostatically with the negatively charged cell surface through the cationic quaternary ammonium head groups; and self-assembles into a micelle/nanoparticle-like structures.
Methods: In vitro studies, including MTT assays and flow cytometry, were conducted using A375 human melanoma cells. Due to the low solubility of the drugs in aqueous medium, DMSO had been used as a solvent previously. However, it caused significant cell death to the A375 cells. To improve the cellular uptake of these lipophilic molecules and eliminate the use of DMSO , CD and CDgemini formulations were used as delivery agents.
Results: Based on physicochemical parameters, we developed a schematic model for the potential interaction of the drug with the delivery agent. The drug formulation IC_{50} values in A375 melanoma cells (1-8 μM) were significantly lower than melphalan, the currently used drug for the treatment of in-transit melanoma. CDgemini formulations showed excellent cellular selectivity, causing cytotoxicity in the A375 cell line while showing no cytotoxic effect to healthy human epidermal keratinocytes.
Flow cytometry was used to monitor cell death and the effect of the CD and CDgemini-encapsulated drug on the cell cycle at IC_{50} concentrations. Both drug formulations showed similar behavior causing the majority of the cells to accumulate in the G_0/G_1 phase and inducing apoptosis.
Conclusion: Our curcumin analogue and novel drug delivery system were effective in selectively inducing apoptosis in A375 melanoma cells at lower dosage than the current standard therapeutic agent, melphalan. We will further develop this novel nanoparticulate therapy for in-transit melanoma metastasis which lacks adequate treatment to date.

39. A Novel Nanoparticle Formulation Overcomes Multiple Types of Membrane Efflux Pumps in Human Breast Cancer Cells

Preethy Prasad[1], Ji Cheng[1], Adam Shuhendler[1], Andrew M. Rauth[2], Xiao Yu Wu[1, 3]. [1]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario; [2]Division of Experimental Therapeutics, Ontario Cancer Institute, Toronto, Ontario, Canada

Purpose: Multidrug resistance in cancer cells is multifactorial involving overexpression of multiple types of membrane drug efflux pumps in addition to other drug resistance mechanisms. Hence inhibition of a single resistance mechanism may not be efficient in achieving therapeutic effects. The objective of the present study was to evaluate the potential of a new polymer lipid hybrid nanoparticle (PLN) system to overcome multiple membrane efflux pumps and enhance doxorubicin (Dox)-mitomycin C (MMC) toxicity against multidrug resistant (MDR) breast cancer cells.
Methods: In vitro cellular uptake, intracellular trafficking, and cytotoxicity of PLN formulations were investigated using the human breast cancer cell lines MCF7/VP overexpressing multidrug resistance protein 1 (MRP1) and MCF7/MX overexpressing breast cancer resistance protein (BCRP). A Clonogenic assay was employed to determine the efficacy of Dox, MMC or Dox-MMC loaded PLN in the MRP1 and BCRP expressing cells and in wild type MCF7 cells. Median effect analysis was conducted to determine the combined action of Dox and MMC.
Results: A synergistic effect of Dox and MMC was observed in all cell lines. Treatment of MDR cells with PLN encapsulating anticancer agents resulted in significantly enhanced cell kill compared with free Dox or MMC solutions at equivalent doses. Dox-MMC co-loaded PLN were effective in killing MDR cells at 20-30 fold lower doses than the free drugs. Co-encapsulation of dual agents into a nanoparticle

was more effective in killing MDR cells than the application of single agent-containing PLN. Fluorescence microscopic images showed perinuclear localization of pyrene loaded PLN in all cell lines.

Conclusion: These results are in agreement with our previous observation on P-gp overexpressing MDA MB 435 breast cancer cells and suggest that the PLN system can overcome multiple membrane efflux pumps and increase cytotoxicity against multidrug resistant breast cancer cells at a significantly lower dose than free drugs.

40. Formulation, Evaluation and Optimization of Pectin–bora Rice Beads for Targeted Drug Delivery to the Colon

K.H. Ramteke, L.K. Nath, A. Bhattacharya. SND College of Pharmacy, Yeola, Dist- Nashik, Dibrugarh University, Dibrugarh, Assam.

Purpose: The aim of research work was formulation and optimization of hydrogel beads of pectin with varying proportion of bora rice so as to control the release of glipizide in the colon.

Methods: Pectin-Bora rice beads were prepared by ionotropic gelation technique by using various proportion of cross linking agents. Drug release from beads was studied by in vitro method. Antihyperglycemic study and gamma scintigraphy was studied by in vivo methods in rats.

Results: Drug release was controlled and followed Korsmeyer and Peppas model. *In vitro* drug release study of optimized formulation P9 provided controlled release of glipizide about 91.06 ± 1.25% within 24 hours which was found to be better as compared to other formulations. Response surface methodology was used for optimization of formulation. Gamma scintigraphy study demonstrated that the beads were intact in the hostile environment of stomach but whenever they reached the colonic region they started disintegration due to the action of anaerobic bacteria present in the colon.

Conclusion: The research work revealed that the bora rice polysaccharide can be used as the carrier for colon targeting drug delivery system. The excellent results were obtained when bora rice was combined with pectin. Hence bora rice powder can be used as a suitable candidate for colon targeted drug delivery system.

41. Development of Amphotericin-B Encapsulated Liposomal Dry Powder Inhaler using Supercritical Fluid Technology for Safe and Effective Treatment of Pulmonary Aspergillosis

Nirav Khatri, Sachin Naik, Deepa Patel, Ambikanandan Misra*. Pharmacy Department, Faculty of Technology and Engineering, Kalabhavan, The Maharaja Sayajirao University of Baroda, Vadodara-390001, Gujarat State, India. *Corresponding author

Purpose: The purpose of present investigation was to develop a procedure for preparation and characterization of Liposomal Dry Powder Inhaler (LDPI) using supercritical carbon dioxide as an antisolvent and assessment of pharmacodynamic effect in suitable animal model.

Methods: The formulation was developed using super critical fluid technology and process was optimized with different parameters viz. temperature, pressure, and flow rate of CO_2. Amphotericin-B (AMP-B) encapsulating LDPI was prepared using hydrogenated soya phosphotidylcholine, cholesterol and antioxidant along with micronized Lactose as a carrier. The optimized AMP-B encapsulated LDPI was characterized for particle size, zeta potential, surface morphology, entrapment efficiency, water content, angle of repose, tapped density and in-vitro drug release. The aerosol performance of AMP-B encapsulated LDPI was carried out using Cascade Impactor. Pharmacodynamic study was performed in Pulmonary Aspergillosis induced Female albino rats, using *Aspergillus fumigatus* isolated from an immunocompromised patient, by administration of LDPI (0.125mg/kg, 0.250 mg/kg of AMP-B) via pulmonary route and compared with nano-liposomal AMP-B suspension (2mg/kg of AMP-B) administered via intra venous (IV) route.

Results: AMP-B encapsulated LDPI was successfully prepared by supercritical fluid technique. The process and formulation parameters were optimized to achieve maximum drug entrapment efficiency and better aerosol performance. Particle size, zeta potential, entrapment efficiency, water content, angle of repose and tapped density were found to be 9.53±0.51µm, 18.9±0.74mV, 82.31±1.71%, 0.82±0.03%, 32.31±0.34° and 0.189 ±0.05 g/cm^3 respectively. The Mean aerodynamic diameter (MMAD), Fine particle fraction (FPF) and % emission were found

to be 5.26 ±0.84 μm, 44.16 ±1.02% and 72.68 ±1.04% respectively. The AMP-B encapsulated LDPI were found to be spherical with smooth surface as seen in photomicrographs of scanning electron microscopy. In-vitro drug release of AMP-B from LDPI was found significantly sustained than AMP-B solution containing deoxycholate. *In-vivo* Pharmacodynamic study suggested efficacy of the AMP-B encapsulated LDPI with respect to IV nano-liposomal AMP-B suspension. LDPI with high dose, of AMP-B, 0.250 mg/kg, had more efficacy than the IV AMP-B nano-Liposomal suspension with the dose of 2mg/kg.

Conclusion: In this study, supercritical fluid technology was successfully used to prepare spherical LDPI with high drug encapsulation and LDPI is used at lower concentration of AMP-B for pulmonary Aspergillosis with local accumulation, which may improve therapeutic efficacy.

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Wednesday, May 25, 2011

42. Thiopurine Methyltransferase and Inosine Triphosphate Pyrophosphohydrolase Genotypes among Patients with Inflammatory Bowel Disease Treated with Azathioprine

Aljudaibi B[1,2], Schwarz UI[1], Urquhart BL[1], Dresser GK[1], Gregor JC[2], Ponich T[2], Chande N[2], Kim RB[1]. [1]Division of Clinical Pharmacology & [2]Gastroenterology, Department of Medicine, Schulich School of Medicine and Dentistry at the University of Western Ontario.
Corresponding Author: baljuda@uwo.ca
Conflict of Interest: None declared

Background: Azathioprine (AZA) is a well established treatment option for patients with inflammatory bowel disease (IBD, ulcerative colitis or Crohn's disease). However, in clinical practice AZA is not always effective, and nearly 20% of patients discontinue AZA due to adverse events. Functional polymorphisms of thiopurine methyltransferase (TPMT) and inosine triphosphate pyrophosphohydrolase (ITPA), two enzymes involved in thiopurine metabolism, have been previously associated with toxicity. It has been proposed that known TPMT and ITPA variant carrier status may predict clinical response and toxicity in IBD patients treated with AZA.
Aims: To determine if TPMT and ITPA genotype is a predictor of clinical response and adverse effects (myelotoxicity, hepatitis, pancreatitis, diarrhea and myalgia) among patients with inflammatory bowel disease treated with azathioprine.
Methods: Patients diagnosed with IBD undergoing AZA therapy or those previously treated with AZA were enrolled. Adverse effects and clinical response were evaluated and correlated with TPMT and ITPA genotypes. Genotyping of previously described polymorphisms including TPMT 238G>C (*2 allele), TPMT 460G>A and 719A>G (*3A allele), as well as ITPA 94C>A, was performed by TaqMan Real-time PCR and polymerase-chain reaction-restriction fragment length polymorphism (PCR-RFLP). The TPMT wild-type allele was designated TPMT*1.
Results: A total of 56 patients were enrolled in the study, with 38 patients (67.9%) on therapy and 18 patients (32.1 %) currently off treatment. Among those, 16 patients (28.6%) responded to AZA therapy, 12 patients (21.4%) partially responded and 15 patients (26.8%) did not respond to therapy. Among 17 patients who experienced adverse events, 10 were unable to tolerate AZA therapy. Three patients (5.4%) experienced severe myelosupression (WBC< 2.0 or neutrophils <1.0). 4 out of 5 heterozygous carriers for TPMT*3A developed adverse events compared to 13 out of 48 wild-type carriers (80% vs 27%, P = 0.032). 2/4 heterozygous carriers for TPMT*3A responded well to therapy compared to 14/48 of wild-type (50% vs 29%, P= 0.58). No TPMT*2 was detected. 3/7 heterozygous patients for ITPA 94C>A developed adverse events compared to 14/46 of wild-type patients (43% vs 30%, P = 0.67), and 1/7 heterozygous patients for ITPA 94C>A were responders to AZA therapy compared to 15/46 of wild-type patients (14% vs 33%, P = 0.66).
Conclusion: Our result suggests that genotyping for TPMT may help predict clinical response and adverse events among patients initiated on AZA therapy. However, there was no correlation between clinical effectiveness and adverse events among patients carrying the ITPA polymorphism.

43. The Effect of Patient Education on the Incidence of CNS Depression in Neonates of Breast Feeding Mothers Taking Codeine: Preliminary Analysis

Chaudhry S[1], Madadi P[1], Lausman A[2], Berger H[2], Gideon Koren G[1]. [1]Division of Clinical Pharmacology and Toxicology, and Motherisk Program; Hospital for Sick Children, [2] Division of Obstetric and Gynecology, St. Michael's Hospital.
Corresponding Author: mueez3@hotmail.com
Conflict of Interest: None declared

Background: Previously, we reported CNS depression and one fatality in breastfed neonates of

codeine-prescribed mothers. This has led us to develop guidelines which are now routinely given to breast feeding mothers taking codeine containing analgesia.

Objectives: We undertook this study to evaluate the effect of patient education on the incidence of CNS depression in neonates of breastfeeding mothers taking codeine as compared to our previous studies.

Methods: This prospective cohort study was conducted between December 2009 and January 2011 at St. Michael's Hospital, in Toronto Canada. The breastfeeding mothers taking codeine for postpartum pain relief following Caesarian section were educated by the study coordinator on the mechanism of action of codeine, its possible side effects in mothers, and what signs and symptoms should be monitored in neonate, and recommended duration of codeine use (four days). Mothers also had 24 hour access to the study physician in case of concern. Telephone follow up was conducted after 7 days.

Results: Out of 220 participants recruited and educated only 2 (0.9%) reported adverse drug reactions in their infants, as compare to 17 (23.6%) out of 72 (P = 0.00005). It is twenty fold lower than the previous report. Demographic characteristics like maternal age, parity, gestational age and fetal weight were not different between the two groups.

Conclusions: Patient education regarding the maximum duration of codeine use and symptoms of possible adverse reactions, including what actions to take, significantly decreases the CNS depression in infants of breast feeding women taking codeine containing medications.

44. Hypertonicity-induced Expression of Human CYP3A in a Humanized Transgenic Mouse Model

Chuang AI, Yang MD, Dalvi P Harper PA, Ito S. Division of Clinical Pharmacology & Toxicology, Hospital for Sick Children, Departments of Paediatrics and Pharmacology, University of Toronto, Physiology and Experimental Medicine program, Research Institute, Hospital for Sick Children.

Corresponding Author: andrew.chuang@utoronto.ca

Conflict of Interest: None declared

The Nuclear Factor of Activated T-cells 5 (NFAT5) mediates hypertonicity-induced human CYP3A expression *in vitro* through an enhancer sequence located within intron 2 of CYP3A7. In order to characterize this phenomenon *in vivo*, we used a humanized transgenic mouse model that contained the entire CYP3A4 and CYP3A7 as transgenes, including introns, 5' and 3' regulatory elements. First, we observed that neither human CYP3A4-3A7, nor known Nfat5 target genes were induced by acute intestinal hypertonicity (12h) or acute systemic intra-vascular hypertonicity (6h), suggesting time-dependency of Nfat5 activation *in vivo*. We then employed prolonged hypertonic conditions in the mouse model: a) intestinal hypertonicity (one week of 8% high-salt diet); and b) prolonged dehydration (one week of dehydration by cycling 24h water deprivation and 24h water ad-lib recovery phases). Stronger Nfat5 protein expression was observed in the duodenum after intestinal hypertonicity, and in the liver and kidney after prolonged dehydration. Consequently, Nfat5 target gene expression was increased, which is consistent with hypertonicity-induced Nfat5 activation. Human CYP3A4 transgene expression also increased in the duodenum after intestinal hypertonicity (4.7±0.9 fold compared to low salt-diet [0% NaCl]: M±SEM; n=6-12, $p<0.05$), and in the liver (10.8±4.0 fold; n=4-14, $p<0.05$) and kidney (2.5±0.5 fold; n=4-10, $p<0.05$) after prolonged dehydration. Furthermore, human CYP3A total protein and activity were increased in these tissues. Our findings indicate ambient hypertonicity-induced human CYP3A4 expression *in vivo*, suggesting a novel mechanism of human CYP3A4 expression control.

45. Chronic Hypertension in Pregnancy: Perinatal Outcome in Women Exposed or Unexposed to Antihypertensive Medications

Matok I[1], Orbach H[2], Gorodischer R[3,4,5], Koren G[1,8,9], Sheiner E[6], Wiznitzer A[6], Levy A[7,9]. [1]The Motherisk Program, Department of Clinical Pharmacology, Hospital for Sick Children, Toronto, Canada, [2]Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel, [3]Departments of pediatrics, Faculty of Health Sciences, Ben-Gurion University of the Negev, [4]Soroka Medical Center, [5]Clalit Health Services (Southern District), [6]Department of Obstetrics and Gynecology, Beer-Sheva, Israel, [7]Epidemiology and Health Services Evaluation, Faculty of Health Sciences, Ben-Gurion University of the Negev, [8]The University of Toronto, Toronto, Canada, and [9]BeMORE collaboration (Ben-Gurion Motherisk

Obstetric Registry of Exposure collaboration).
Corresponding Author: ilan.matok@sickkids.ca
Conflict of Interest: None declared

Objective: Despite high rates of hypertension disorder during pregnancy, the effects of hypertension on perinatal outcome have not been appropriately separated from those of the medications used. We evaluated the safety of exposure to antihypertensive medications during pregnancy, when accounting for untreated hypertension.
Study Design: A population- based retrospective cohort study was performed, comparing all pregnancies of women exposed and not exposed to antihypertensive medications during pregnancy. A computerized database of medications dispensed from 1998 to 2008 was linked with computerized databases containing maternal and infant hospitalization records from the district hospital during the same period. Multiple logistic regression models were performed to control for confounders.
Results: During the study period 100,029 deliveries occurred; of those, 620 (0.6%) were exposed to at least one antihypertensive medication (methyldopa or atenolol) during pregnancy. A higher rate of low birth weight newborns (LBW<2500 grams, 24.4% vs. 10.3%; p<0.001), intrauterine growth restriction (IUGR, 5.2% vs. 2.2%; p<0.001) and preterm delivery (PTD<37 weeks, 24.4% vs. 8.1%; p<0.001) were noted among pregnancies of women who were exposed to antihypertensive medications during the third trimester of pregnancy, as compared to women without hypertension and not exposed to antihypertensive medications. The association between antihypertensive medications (in general), methyldopa and atenolol, and LBW, IUGR and PTD remained significant after adjusting for maternal age, ethnicity, smoking, diabetes mellitus, lack of prenatal care, multiple pregnancy and parity (OR=3.7 95%CI:2.9-4.8; OR=4.3 95% CI:3.0-6.3; OR=3.7, 95%CI: 2.9-4.8 respectively). Nevertheless, a similar association was noted while comparing untreated woman with chronic hypertension during pregnancy (n=1074) to woman without chronic hypertension and not exposed to antihypertensive medications (n=97,820) (OR=1.7 95%CI: 1.4-2.0, OR=2.1, 95%CI:1.5-2.9, OR=1.9; 95%CI:1.6-2.3 for LBW, IUGR and PTD, respectively)
Conclusion: Chronic hypertension with or without treatment during pregnancy is an independent and significant risk factor for adverse perinatal outcomes such as LBW, IUGR and PTD as compared to births of women without chronic hypertension and without exposure to antihypertensive medications.

46. Intracellular mechanism Involved in Downregulation of Hepatic Cytochrome P450 by Chronic Renal Failure and Parathyroid Hormone

Michaud J[1,2], Naud J[1,2], Beauchemin S[1], Leblond FA[1],Pichette V[1,2]. [1]Centre de recherche de l'hôpital Maisonneuve-Rosemont, Montréal, Québec, Canada; [2]Département de pharmacologie, Faculté de Médecine, Université de Montréal. Corresponding Author: josee_michaud@hotmail.com
Conflict of Interest: None declared

Background: Chronic renal failure (CRF) is associated with a decrease in drug metabolism, due to a down-regulation of hepatic cytochrome P450. Previous studies indicated that CRF modifies activity, protein and mRNA expression of different P450 isoforms *in vivo* and *in vitro* via circulating mediators. Parathyroid hormone (PTH) was identified as one of them. CRF and PTH cause an inhibition of P450. The mechanism remains to be defined.
Objective: The aim of this study was to evaluate the contribution of different signaling factors like; PXR, CAR, NF-κB, PKA and PKC.
Methods: Four groups of rats were studied CTL, CRF, CRF with parathyroidectomy (CRF-PTX) and CTL-PTX. Liver and hepatocyte CAR and PXR mRNA expression was measured by qPCR and their protein expression was measured by Western blots. Cultured hepatocytes were incubated with CTL, CRF, CRF-PTX and CTL-PTX sera, or with or without PTH, and nuclear extracts were obtained. Nuclear extracts were used for NF-κB flux cytometry and Western blots (p50 and p65).
Results: We observed down-regulations of PXR and CAR protein (43%, 44%, respectively) and mRNA (40%, 42%, respectively) CRF rats liver. PTX prevents the mRNA down-regulation of CAR and PXR in CRF rats. We observed a NF-kB accumulation in liver's nuclei of CRF rats. Furthermore, blocking NF-κB with inhibitors counteracts the effect of CRF and PTH on P450 expression.
Conclusion: In conclusion, CAR, PXR and NF-κB could be implicated in the down-regulation of hepatic P450 by CRF and PTH.

47. Quantitative In Vitro to In Vivo Prediction of P-glycoprotein-mediated Drug Transport

Morgan AD, Kim RB, Tirona RG. Department of Physiology and Pharmacology; Department of Medicine, Division of Clinical Pharmacology, Lawson Health Research Institute, The University of Western Ontario, London, Ontario, Canada.
Corresponding Author: amorga@uwo.ca
Conflict of Interest: None declared

Introduction: It is well appreciated that membrane transporters play important roles in drug disposition, a critical determinant of the pharmacological and toxicological profile of all drugs. P-glycoprotein (P-gp), encoded by the ABCB1 gene, is a clinically important and well-characterized efflux transporter affecting drug absorption, distribution and elimination. Despite that a number of in vitro assays for P-gp activity are commonly used, the in vivo relevance of data derived from such assays remains unclear due to both incomplete knowledge of transporter intrinsic clearance and a lack of predictive extrapolation strategies. Here we provide the theoretical and experimental strategy as well as preliminary data for in vitro to in vivo prediction of P-gp mediated transport.
Methods: We have selected sitagliptin, an unmetabolized drug used in Type 2 diabetes as a P-gp probe drug. P-gp transport of sitagliptin was examined in a model of cultured, polarized epithelial cells heterologously expressing varying amounts of transporter.
Results: By monitoring sitagliptin transcellular flux using liquid chromatography – tandem mass spectrometry in combination with mathematical modeling, we obtain a value for P-gp intrinsic clearance. This intrinsic transport clearance is normalized to P-gp protein content of cells as determined by quantitative proteomic analysis.
Conclusion: These data, which would be the first of their kind, are expected to form the foundation for quantitative methods for in vitro to in vivo prediction of drug pharmacokinetics and ultimately therapeutic efficacy.

48. A Comparison of Folic Acid Pharmacokinetics in Obese and Non-Obese Women of Childbearing Age

Stern SJ[1,2], Matok I[2], Kapur B[1,2,3,4], Koren G[1,2]. [1]Department of Pharmacology, University of Toronto; [2]Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children; [3]Department of Clinical Pathology, Sunnybrook Health Science Centre; [4]Department of Laboratory Medicine and Pathobiology, University of Toronto.
Corresponding Author: seth.stern@utoronto.ca
Conflict of Interest: None declared

Background: While maternal folate deficiency during the periconceptional period represents a major risk factor for neural tube defects (NTD), obesity has been recognized as an additional risk factor. Studies have identified an increased risk for NTD-affected births among obese mothers even after adjusting for folic acid supplementation. However, while folic acid intake may have been at the recommended dose in these samples, blood folate concentrations were not monitored to ensure protective levels were reached.
Objectives: To compare folic acid pharmacokinetics in obese and non-obese women of childbearing age.
Methods: Healthy obese (n = 12) and non-obese (n = 12) women of childbearing age volunteered to participate. Each obese participant was matched to a non-obese participant, and assigned an equivalent dose per kilogram body weight of folic acid. Folic acid was orally administered after a 6-hour fast, and blood samples were taken over a 10-hour period to evaluate pharmacokinetic parameters.
Results: Area under the curve (AUC) was found to be significantly higher in the obese group (P = 0.008). Defining AUC as a function of dose per lean body weight (LBW) was found to be a stronger predictor than dose per total body weight (r^2 = 0.90 and 0.76, respectively).
Conclusions: This indicates that the body tightly controls systemic exposure to folic acid, with 90% of variability in AUC controlled by the dose per LBW. Periconceptional supplementation recommendations may need to be adjusted to account for LBW differences in the obese population.

49. Examination of Superparamagnetic Iron Oxide Nanoparticle Accumulation and Toxicity in Various Brain Related Cell Culture Models

Sun Z, Yathindranath V, Hegmann T, Miller DW. Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada

Corresponding Author: sam.zhizhisun@gmail.com
Conflict of Interest: None declared

Background: Superparamagnetic iron oxide nanoparticles (IONPs) have shown great promise in biomedical imaging and drug delivery. However, issues pertaining to the toxicity of IONPs are a concern especially for those applications involving IONPs and the brain.
Objectives: To determine whether cell toxicity was correlated with cell accumulation of various IONP formulations and to examine the impact of magnetic field on both these parameters.
Methods: Bare, oleic acid and bovine serum albumin (BSA) coated IONPs were examined in a mouse brain microvessel endothelial cell line (bEnd.3) and mouse primary cultured neurons and astrocyte preparations in the presence and absence of a magnetic field. Accumulation of IONPs was examined over a 2 hour period using Prussian blue staining. Cytotoxicity was assessed by addition of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) after 24-hour exposure to various concentrations of formulated IONPs.
Results: In neurons, oleic acid coated IONPs had the greatest cell association compared to BSA coated IONPs. In contrast, rank order of cell association for IONPs in astrocytes was uncoated > oleic acid > BSA coated. The presence of magnetic field resulted in more IONPs within the cells regardless of formulation. None of the formulations produced significant (i.e. greater than 10%) toxicity at concentration up to 100ug/mL. Neurons appeared more sensitive to oleic acid coated IONPs at concentrations above100 ug/mL.
Conclusions: The various formulations of IONPs were safe in all cells examined at concentrations less than 100 ug/mL. Accumulation and cytotoxicity of IONPs was increased in the presence of a magnetic field.

50. Down-regulation of Human Cytochrome P450 2C8 by 3-methylcholanthrene

Utgikar R, Riddick DS. Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario.
Corresponding author: rucha.utgikar@utoronto.ca
Conflict of interest: None to declared

Background: 3-Methylcholanthrene (MC) is a model polycyclic aromatic hydrocarbon that induces cytochrome P450 1A1 (*CYP1A1*) expression via aryl hydrocarbon receptor activation. MC also down-regulates certain constitutive hepatic P450s in rodents, but the mechanism and human relevance of this response remain poorly understood. Recent reports suggest that MC down-regulates the expression of *CYP2C8* in primary human hepatocytes. This has potential clinical importance because human liver CYP2C8 metabolizes therapeutic agents, such as the antineoplastic paclitaxel, and endogenous signalling molecules, such as all-*trans*-retinoic acid.
Objectives: To determine if MC alters the expression of *CYP2C8* at the mRNA level in two human hepatocellular carcinoma cell lines, HepG2 and HepaRG.
Methods: Cells were treated with vehicle or MC (1 or 5 μM) for 24 or 48 h. Cytotoxicity was assessed by trypan blue dye exclusion. CYP1A1 and CYP2C8 mRNA levels were measured by real-time RT-PCR.
Results: Under conditions that resulted in minimal cytotoxicity, MC induced CYP1A1 mRNA levels in HepG2 cells by approximately 245- to 425-fold. MC at 5 μM suppressed CYP2C8 mRNA levels in HepG2 cells by approximately 78% at 24 h and this response did not persist at 48 h. Similar studies are in progress using HepaRG cells as a more differentiated model that maintains higher basal levels of several constitutive P450s.
Conclusions: The demonstration of CYP2C8 mRNA suppression by MC in human liver-derived cell lines will facilitate our efforts to define the molecular mechanisms and functional impacts of the modulation of this important human P450 by environmental toxicants.

51. Hepatic Drug Metabolism in Varying Degrees of Renal Function using Rat Models of Renal Failure

Velenosi TJ[1], Fu A[1], Luo S[2, 3], Wang H[2, 3], Urquhart BL[1, 3]. [1]Schulich School of Medicine and Dentistry, Department of Physiology and Pharmacology, The University of Western Ontario, London, Ontario, Canada; [2]Department of Surgery, The University of Western Ontario, London, Ontario, Canada; [3]Lawson Health Research Institute, London, Ontario, Canada. Corresponding Author: tvelenos@uwo.ca
Conflict of Interest: None declared

Background: Chronic renal failure (CRF) is the result of decreasing renal function over time. Expression and activity of drug metabolizing enzymes such as CYP3A are decreased in end stage renal disease. However, only a small percentage of patients with CRF are at the final stage of the disease.
Objectives: This study aimed to determine the changes in drug metabolizing enzyme function and expression in rats with varying degrees of renal failure.
Methods: Sprague-Dawley rats underwent either 2/3 or 5/6 nephrectomy by partial left kidney resection followed by complete right nephrectomy. Control rats underwent sham laparotomies. Rats were sacrificed on day 42 and CYP3A activity was determined in liver microsomes by evaluating midazolam metabolism using ultra-performance liquid chromatography with photodiode array detection.
Results: On day 42, serum creatinine levels were 23.0 ± 1.4, 37.4 ± 1.0 and 75.4 ± 14.7 μM in control, 2/3 and 5/6 nephrectomized rats, respectively. V_{max} values for 4-OH and 1-OH midazolam formation were lower in 2/3 nephrectomized rats (29% and 45%, respectively) and 5/6 nephrectomized rats (36% and 48%, respectively) compared to controls (P < 0.05). CYP3A2 protein expression was significantly decreased in both experimental groups compared to controls (P<0.05). Vmax values for midazolam metabolism were weakly correlated with day 42 serum creatinine levels (P < 0.05).
Conclusions: Our results demonstrate that CYP3A activity and expression is decreased in mild renal failure, which suggests that drug therapy for patients in early stages of kidney failure may be compromised for drugs that are substrate for CYP3A.

52. Mechanism of Convolvulus Arvensis Induced Relaxation of Thoracic Aorta in Rabbit

Al-Aghawani W[1], Al-Madi S[2], AL-Lahham A[2], Abu Jayyab A[1]. [1]Faculty of Pharmacy, International University for Science & Technology, Syria; [2]Faculty of pharmacy, Damascus University, Damascus, Syria.
Corresponding Author: jayyab@yahoo.com
Conflict of Interest: None declared

Background: *Convolvulus arvensis* L plant (CAL) L is shown to produce relaxation in some smooth muscle. It is used in traditional herbal medicine for many purposes, especially as anti-spasmodic in Middle East.
Objective: Experiments were undertaken to determine whether ethanolic extract obtained from CAL has vasorelaxant activity in the rabbit aorta rings and, if so, to elucidate the underlying mechanism.
Methods: Rabbit aorta rings were suspended in organ chambers for the measurement of changes in isometric tension in the presence of pheylephrine or glibenclamide.
Results: CAL decreased vessels contraction by phenylephrine in *both presence* and *absence* of an intact *endothelium* groups compared with the control group (IC_{50} = 358 mg/ L with endothelium and 399 mg/ L without endothelium, P< 0.05). In addition, pretreatment of aortic rings with glibenclamide inhibited partially CAL induced relaxation in Phenylephrine–contracted aortic tissues.
Conclusions: These data indicate that calcium might play an important role in the mechanism of CAL induced relaxation of aorta tissues. Also, it might be involved, at least in part, potassium channel in the relaxation activity of CAL. The present findings suggest that CAL could be a candidate of herbal medicine for cardiovascular diseases associated with aorta tissues dysfunction.

53. In Vitro Metabolism Study of Specific Cyp450 Substrates in Breast Cancer Cell Lines

Armstrong C,[1,2] Belanger F,[2] Gaudette F, [2] Balicki D,[3] Turgeon J[1,2]. [1]Faculty of Pharmacy, Université de Montréal, Montreal, QC, Canada ; [2]CRCHUM, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, QC, Canada; [3]Faculty of Medicine, Université de Montréal, Montreal, QC, Canada. *Corresponding author:* jacques.turgeon.chum@ssss.gouv.qc.ca
Conflict of interest: No conflict of interest to report

Background and Objectives: A treatment failure is commonly observed in breast cancer patients due to an innate or acute resistance to anti-cancer agents. Understanding, the local metabolism of anti-cancer agents by CYP450s in breast cancer cells could help in the development of a more targeted treatment approach. Our goal is to evaluate if measurable metabolism of specific CYP450 substrates is present in breast cancer cell lines, and to compare this

metabolism to the expression of CYP450 mRNAs.
Method: Seven commonly used breast cancer cell lines (and one normal breast cell line) were cultured and then plated in 24 well plates. Once confluent, the cells were incubated in the presence of specific substrates, and the metabolism was determined by measuring the appearance of metabolites by LC-MS-MS. In order to correlate metabolism with mRNA expression, RNA was isolated from each cell line, and the mRNA relative expression was determined by RT-PCR (TaqMan Assay) for 19 CYP450 isoforms.
Results: RT-PCR results demonstrated that each cell line showed differential expression of CYP450 mRNAs. The expression of CYP2J2 is of interest because several cell lines showed elevated expression of this isoform, such as MCF-7 cells, whereas others, such as Hs578T cells, showed little expression. Metabolism was observable in cells lines for the specific CYP2J2 substrate, Ebastine, where MCF-7 cells demonstrated measurable metabolism in as little as 30 minutes.
Conclusions: Our results suggest that the local metabolism of various anti-cancer agents could be significant enough to greatly impact the resistance that is observed in breast cancer patients.

54. A Systematic Review of the Fetal Safety of Interferon Alfa

Yazdani Brojeni P, Matok I, Garcia Bournissen F, Koren G. Motherisk Program, Hospital for Sick Children, University of Toronto.
Corresponding Author: mpyazdani@yahoo.ca
Conflict of Interest: None declared

Background: Interferon alpha (INF) is an effective treatment for a variety of conditions. Since these conditions could happen in pregnancy, Information regarding the safe use of this medication in pregnancy is essential. This systematic review attempts to summarize all published data on outcomes of INF alpha exposed pregnancies.
Methods: Using key words INF alpha, pregnancy, we searched Pub Med, EM BASE, and Google, since INF alpha was introduced in the market. We were able to locate only case reports of INF alpha exposure in pregnancy. All cases were collected and included in our review. We also collected 71 cases that were diagnosed with essential thrombocythemia but did not receive any medication in pregnancy.
Results: Among 61 INF alpha exposures in pregnancy we located, mean maternal age was 31±4 years, median 33 years, and range 23-43 years. Mean full term babies' weight was 3010, median 2680 grams and the range 1350-3800. Mean gestational age at delivery was 37±3 weeks, median 38, range 30-41 weeks. No major malformation or stillbirth was reported. There was one spontaneous abortion, and 12 preterm deliveries (20 % of all the cases). In 71 cases with the same underlying diseases who did not receive any medication in pregnancy, 46 out of 71 had early or late abortion. There were 3 cases of stillbirth and 4 cases experienced preterm delivery. 18 cases had term normal babies.
Conclusion: This systematic review suggests that INF alpha does not increase the risk of abortion, major malformation, still birth, and premature deliveries, and probably it has a protective effect on prevention of early/late spontaneous abortion and still birth in this particular population.

55. The Motherisk Cancer in Pregnancy Forum: A Unique Way of Counseling

Carey N[1,2]; Koren G[1,2,3]. [1]Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, Toronto, Canada; [2]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Canada; [3]Ivey Chair in Molecular Toxicology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Canada.
Corresponding Author: nat.carey@utoronto.ca
Conflict of Interest: None declared

Background: Cancer occurs in approximately 0.07-0.1% of pregnancies. The diagnosis of cancer in pregnancy complicates optimal treatment due to potential risk to the fetus. The Motherisk program established The Consortium of Cancer in Pregnancy Evidence (CCoPE) in an effort to address the deficiency in information regarding the care of cancer and associated therapies in pregnancy. Subsequently, a Cancer in Pregnancy Forum was created to provide unique, evidence-based counseling to women and their medical professionals.
Objectives: To describe the characteristics of the cases counseled by the Cancer in Pregnancy Forum.
Methods: Review of cases documented in the Cancer in Pregnancy Forum since its inauguration. Data were collected on the person who initiated the

post, the type of cancer, the treatment plan, response time and completeness of information.
Results: There were a total of 129 inquiries on the Cancer Forum from 1999 to 2011. Healthcare providers and scientists initiated 41% of the postings, whilst women or their partners made up the remaining 59%. Inquiries regarding maternal chemotherapy were the most frequent (30%), followed by questions concerning specific cancers (20%), radiation (17%), paternal chemotherapy (13%), other therapies (12%), breastfeeding (5%), female fertility (1.5%) and male fertility (1.5%).
Conclusions: A diagnosis of cancer during pregnancy is a major stress for the expecting mother and her family. The Cancer in Pregnancy forum is the only forum of its kind worldwide, providing women and medical professionals evidence-based information regarding diagnosis, treatment, symptoms and other concerns with respect to cancer during pregnancy and lactation.

56. Ototoxicity in Mexican Children Receiving Cisplatin Based Chemotherapy

Castelan-Martinez OD[1,2], Garcia-Rojas Y[3], Fierro-Evans M[3], Marques-Avila S[3], Rivas-Ruiz R[1], Jimenez-Mendez R[4], Castaneda-Hernandez G[2], Carleton, B[4]. [1]Unidad de Epidemiologia Clinica, HIMFG, Mexico; [2]Departamento de Farmacologia CINVESTAV-IPN, Mexico; [3]Servicio de Audiologia y Foniatria, HIMFG, Mexico; [4]Department of Paediatrics, University of British Columbia, Canada.
Corresponding Author: rjimenez@popi.ubc.ca
Conflict of Interest: None declared

Introduction: Cisplatin has been widely used as a chemotherapeutic agent for a variety of paediatric malignancies. One of the most incapacitating adverse drug reactions reported on patients under cisplatin therapy is ototoxicity, associated with permanent bilateral hearing loss. Even though extensive publications are available in the literature, information on Latin-American patients is scare.
Objective: To describe the frequency and severity of ototoxicity in paediatric patients treated with cisplatin based chemotherapy in a third level paediatric hospital in Mexico City.
Methods: Three audiology evaluations were prospectively conducted in paediatric patients (< 18 years) with solid tumour cancers, at baseline and at the end of each of two chemotherapy cycles. Audiology data was used to classify hearing loss according to the Common Terminology Criteria for Adverse Events.
Results: Forty-two patients participated in this study, aged 4 to 17 years, 52% were female, being osteosarcoma (78.6%) the predominant cancer type. Bilateral hearing loss was observed in 31% of patients at the end of the first chemotherapy cycle, while 62% report hearing loss at the end of the second cycle, mainly in frequencies over 4,000hz ($p<0.001$). This represent an increased risk of hearing loss (OR= 2.00, [CI 95% 1.26-3.17], $p=0.005$) from the first to the second cycle of chemotherapy. Cumulative dose, age, treatment scheme and tumour type were not related to hearing lose.
Conclusion: Hearing loss in Mexican children under cisplatin therapy is similar to that reported previously in other populations. Although ototoxicity was related to chemotherapy cycle number, it cannot be positively correlated with cumulative dose of cisplatin, tumour type or age. Further research is required to correctly characterize ototoxicity in Mexican patients.

57. Statins and Their Category X Pregnancy Classification: Pravastatin May Merit Reconsideration for a Novel Indication

Davidovits, J[1,2,3] Laskin, CA[2,3,4] Casper, RF[3,4] Clark, CA[2,4] Spitzer, KA[2,4] Koren, G[1,3]. [1]Hospital for Sick Children; [2]LifeQuest Centre for Reproductive Medicine; [3]University of Toronto; [4]Mount Sinai Hospital. Corresponding Author: judith.davidovits@gmail.com
Conflict of Interest: None declared

Background: Animal models of recurrent pregnancy loss (RPL) and preeclampsia have implicated elevated levels of tissue factor (TF), and resultant hypercoagulability and inflammation, as a key factor in placental and fetal damage and pre-eclampsia. Using a mouse model that shares features with human RPL and pre-eclampsia, treatment with pravastatin (HMG CoA reductase inhibitor) down-regulated TF and rescued pregnancies. However, the FDA has given a category X classification for all statins (i.e. contraindicated in pregnancy). Yet, unlike lipophilic statins that comprise the majority of this drug group, pravastatin is hydrophilic and hepatospecific, and therefore less likely to enter fetal circulation.

Objective: To evaluate the FDA category X classification for pravastatin.
Methods: Literature review of teratogenic effects of pravastatin exposure.
Results: Contraindication of statins in pregnancy is based on animal testing and a small number of case reports involving two lipophilic statins, lovastatin and simvastatin. Increased risk of teratogenicity has not been established. Contrary to lipophilic statins, pravastatin did not exhibit teratogenicity in animal testing or human cases. Pravastatin has not been shown to be teratogenic in multiple cohort studies.
Conclusions: Despite the lack of established teratogenic risks, pravastatin remains contraindicated in pregnancy. Since suspension of hypercholesterolemia therapy during gestation is considered safe, this contraindication has not previously been questioned. However, promising new indications for prevention of adverse pregnancy outcomes and reassuring reports of its safety during gestation support both reevaluation of its contraindication and implementation of controlled clinical studies to establish the efficacy of pravastatin for treatment of RPL and preeclampsia.

58. Switching from Brand-Name to Generic Psychotropic Medications: A Literature Review

Desmarais JE, Beauclair L, Margolese HC. McGill University Health Centre, Clinical Psychopharmacology and Therapeutics Unit, Montreal, Quebec, Canada. Corresponding Author: julie.desmarais@mail.mcgill.ca
Conflict of Interest: None declared

Background: Current world economics encourage use of generic medications which are less expensive than brand name originals. There is however controversy as to their clinical equivalence. Clinical deterioration, adverse effects and toxicity have been described with generic substitution.
Objective: To explore issues about generic substitution of psychotropic medications reported in the literature.
Methods: Pubmed was searched from January 1, 1974 to March 1, 2010. The MeSH term "generic, drugs" was combined with "anticonvulsants", "mood stabilizers", "lithium", "antidepressants", "antipsychotics", "anxiolytics" and "benzodiazepines." Articles in English, French, or Spanish were considered if they discussed clinical equivalence of generic and original medications, generic substitution, or issues about effectiveness, tolerability, compliance, or economics encountered with generics. Additional articles were obtained by searching the bibliographies of relevant references.
Results: Formulation substitution of anticonvulsants/mood stabilizers (carbamazepine, valproate, lamotrigine, gabapentin, topiramate, lithium) antidepressants (amitriptyline, nortriptyline, desipramine, fluoxetine, paroxetine, citalopram, sertraline, mirtazapine, bupropion, venlafaxine) antipsychotics (clozapine, risperidone) and anxiolytics (clonazepam, alprazolam) has been linked to clinical deterioration, decreased tolerability/toxicity and pharmacokinetic changes. Decreased compliance may result from a change in formulation. Generic substitution is not always economically profitable when the consequences of relapses and decreased compliance are taken into account.
Conclusions: Publication bias and heterogeneity of the studies are limitations of this review. It is yet premature to determine the true equivalence between generic and original medications. Caution is still advised when a patient's medications are switched to another formulation. Health professionals should be sensitized to the possible consequences of generic substitution.

59. CYP2C19 Genotyping in two Infants with Gastroesophageal Reflux

Fujii H[1], Sakaguchi S[1], Garcia-Bournissen F[1], Gaedigk A[2], Leeder S[2], Ito S[1]. [1]Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto; [2]Clinical Pharmacology & Therapeutics, Children's Mercy Hospital, Kansas City.
Corresponding Author: hisaki.fujii@sickkids.ca
Conflict of Interest: None declared

Background: Although the impact of CYP2C19 genotypes on PK of proton pump inhibitors (PPI) has been extensively investigated, clinical utility of CYP2C19 genotyping is still not clear. Also the optimal PPI dosage for children under 2 years old has not been yet determined. Here we show 2 infant cases with severe gastroesophageal reflux who failed to respond to high dose of pantropazole.
Case 1; 11 months old (5.6kg). Pantoprazole was administered via continuous infusion 8.5mg/kg/day (standard dose: 1mg/kg/day), but gastric pH

remained low. CYP2C19 genotyping showed that the patient was a heterozygote of CYP2C19 wild type (*1) and high function genotype (*17). Apparent clearance was 0.37L/kg/h (reported clearance at 2-4 y.o; 0.20±0.23 L/kg/h). Pantoprazole was then switched to 15mg tid oral omeprazole with successful therapeutic response.
Case 2; 5 months old (5.4kg). She received 14.0mg/kg/day of pantoprazole without response. Her CYP2C19 genotype was *1/*1 (wild type). Pantoprazole was changed to oral omeprazole with remarkable therapeutic response.
Discussion: These 2 infants required 8.5 times and 14 times higher doses of pantoprazole than normal recommended dose for infants (1mg/kg/day). The high clearance was confirmed in Case 1, which is consistent with CYP2C19*17 genotype. On the other hand, Case 2 suggests a wide range of functionality within the CYP2C19 *1/*1 genotype and/or pharmacodynamics (PD) variations.
Conclusions: CYP2C19*17 genotype may be sufficient but not necessary as a cause of pantoprazole therapeutic failure in infants. Studies of CYP2C19 genotype - pantoprazole PK/PD correlation are needed to determine the optimal dose for infants.

60. The Incidence of Tacrolimus-induced Nephrotoxicty in Children: Do we Really Know?

Gijsen VMGJ[1,2], Koren G[2,3], de Wildt SN[1]. [1]Intensive Care and Department of Pediatric Surgery, Erasmus MC Sophia Children's Hospital, Rotterdam, the Netherlands; [2]Division of Clinical Pharmacology and Toxicology, the Hospital for Sick Children, Toronto, On, Canada; [3]Ivey Chair in Molecular Toxicology, Department of Medicine, University of Western Ontario, London, ON, Canada
Corresponding Author: violettegijsen@gmail.com
Conflict of Interest: None declared

Background: Tacrolimus is a calcineurin-inhibitor and the drug of choice for pediatric solid organ transplants. Although the renal effects of calcineurin-inhibitors have widely been studied, the data on tacrolimus-induced nephrotoxicity in children is limited.
Objectives: To determine the incidence of tacrolimus-induced nephrotoxicity in children by systematically reviewing the literature.
Methods: Pubmed/Medline, Embase and Google were searched from their inception till February 2nd 2011 with the search terms "tacrolimus", "nephrotoxicity", "transplantation" and "children". References of relevant articles were screened as well.
Results: Fifteen of the 87 articles were considered relevant. Ten papers researched liver transplant recipients, 4 kidney transplant recipients and one abdominal and thoracic transplant recipients. The incidence of tacrolimus-induced nephrotoxicity ranged from 0%-76.5%. This range is a direct result of the differences between the studies. Follow-up times ranged from pre-transplant until 8 years post-transplantation. However, five studies did not report their follow-up times. Seven studies did not clarify their definition for tacrolimus nephrotoxicity. Two of these studies did not mention the type of tacrolimus toxicity reported. Those studies reporting a definition for tacrolimus-induced nephrotoxicity, mainly used grading systems based on GFR. One study used histological grading systems. However, cut-offs for nephrotoxicity were not reported in any of the grading systems.
Conclusions: Due to the many differences between the studies, a decisive incidence number cannot be given. Further studies need to address the implications of these different definitions on the incidence number to have a better understanding of the vastness of the problem.

61. Assessing Interactions between Herbal Medicines and Drugs: A Review

Girard L[1], Filippelli A[2], Boon H[3], Gardiner P[4], Vohra S1,5. [1]CARE Program, Department of Pediatrics, Faculty of Medicine, University of Alberta, Edmonton, Alberta, [2]School Of Public Health, Boston University, Boston, Massachusetts, [3]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario; [4]Department of Family Medicine, Boston University Medical Center, Boston; [5]School of Public Health, Department of Pediatrics, and Department of Medicine, University of Alberta, Edmonton, Alberta
Corresponding Author: sabina.vohra@utoronto.ca
Conflict of Interest: None declared

Background: Herbal products are generally considered safe, but not necessarily when used concurrently with drugs. A reference tool for rapid identification of known herbal medicine-drug interactions was created to help clinicians.

Objective: To review the herb-drug literature and update the herb-drug interaction grid.
Methods: Searches were conducted in MEDLINE and EMBASE between 2007 and 2010, using the following search terms: 34 commonly used herbs combined with 'clinical trials', 'case studies', and 'case reports'. Reference lists of relevant review articles were analyzed for additional papers, and a tertiary source was consulted to identify other herb-drug interactions. Data extraction included the amount of evidence regarding the severity and likelihood of the interactions between each herb and each category of pharmaceutical agents. The interactions were classified into four groups: 1) No reported or theoretical interactions, 2) Theoretical interactions based on animal of in vitro data, 3) Theoretical interactions extrapolated from clinical data, and 4) Interactions supported by clinical evidence.
Results: A total of 1553 references were identified by the searches. 1514 articles have been screened, and 120 have been included for extraction. Full results will be ready in October.
Conclusions: The herbal medicine-drug interaction grid will allow clinicians to have a guide on potential harms based on the most recent literature. This quick reference guide should facilitate clinicians' ability to answer questions about the concurrent use of herbal medicine products and prescription medicines, thus enhancing patient safety.

62. Role of Metabolite Receptor GPR91 in Post-Stroke Recovery

Hamel D[2], Sanchez M[3], Rivera JC[2], Hou X[1], Bhosle V[3], Chemtob S[1,2,3]. Hôpital Sainte-Justine Research Center, Department of [1]Pediatrics and [2]Pharmacology, Université de Montréal, Montréal, Québec, Canada; [3]Department of Pharmacology & Therapeutics, McGill University, Montréal, Québec, Canada.
Corresponding Author: david.hamel.1@gmail.com
Conflict of Interest: None declared

Background: Numerous events affecting normal cerebral blood flow can cause hypoxia-ischemia (H-I), which can lead to major neurological disabilities in infants and adults. There is currently a void in treatment modalities to counter cerebrovascular injury. We have recently demonstrated that the Krebs cycle product succinate, links capillary function to tissue metabolic needs via the G protein-coupled receptor (GPCR) GPR91. The functional benefits of this coupling and its effect on post-stroke recovery are still unknown.
Objectives: We assess the role of succinate in orchestrating brain angiogenesis and recovery during post-ischemic events in the newborn.
Methods: To test our hypothesis the Rice-Vanucci model was employed. Succinate levels were evaluated by mass spectrometry at different time-point following H-I. Expression of GPR91 was determined by immunohistochemistry and western blot. Expression of pro-angiogenic genes was evaluated by real-time pcr.
Results: We detected a rapid accumulation of succinate following the insult. GPR91 was primarily localized in neurons within the cerebral cortex. To determine the role of succinate in this injury paradigm, we injected succinate intraventricularly in newborn animals and detected a 30% increase in blood vessel formation which was corroborated *ex vivo* with a robust increase in vessel density in cortical explants. Stimulation of primary neuronal cultures with succinate lead to a 2.1-fold increase in VEGF mRNA.
Conclusion: Collectively our data demonstrate that succinate strongly affects the cerebral vascular response to ischemia. These results offer a new and potentially important target to optimize recovery following cerebral vascular injury.

63. A Drug-drug Interactions Study Between Rosuvastatin and Pantoprazole after Oral Administration in Healthy Male Subjects

Huguet J[1,2], Gaudette F[2], Bélanger F[2], Dallaire S[2,4], Iglesias L[2], Doucet J[2], Nelson M[2], Chiasson JL[2,3,4], Hamet P[2,3], Turgeon J[1,2]. [1]Faculty of Pharmacy, University of Montreal, Montreal, Quebec, Canada; [2]Research Centre, Centre Hospitalier de l'Université de Montréal (CRCHUM), Montreal, Quebec, Canada; [3]Department of Medicine, University of Montreal, Montreal, Quebec, Canada; [4]Research Group on Diabetes and Metabolic Regulation, CRCHUM, Montréal, Québec, Canada
Corresponding Author: jade.huguet@umontreal.ca
Conflict of Interest: On behalf of all authors, cannot identify any potential conflict of interest.

Purpose: Rosuvastatin (ROSU) disposition involved intestinal and hepatic membrane transporters such as OATP1B1, 1B3, 2B1, ABCG2 (BCRP) and NTCP.

Pantoprazole (PANTO) is a potential substrate/inhibitor of ABCG2. Our objective was to select PANTO as a clinical probe to study the impact of ABCG2 inhibition on the pharmacokinetic of ROSU.

Methods: Eight healthy White male subjects performed a single-center, 2-period, cross-over study. They received, on 2 occasions, 7 days apart, either an oral dose of PANTO (40 mg) or placebo in the morning followed by a single oral dose of ROSU (10 mg) 1 hour after. The next morning (24 hours after), subjects were given a 2nd 40 mg dose of PANTO. Genotyping for 3 single nucleotide polymorphism *ABCG2* C421A, *SLC01B1* T521C and A388G (OATP1B1) was performed. Blood samples were collected before and 0.25, 0.5, 0.45, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 26, 28, 30, 32, 48 and 72 hours after ROSU administration. Urine was collected over 72 hours. Plasma and urine samples were analyzed by LC/MSMS. Data were analyzed by ANOVA with repeated measures adjusting for sequence and period for Cmax, AUC, CL/F and CLr. No effect boundary of 80-125% for the point estimates and the 90% confidence interval (CI) for the ratio of the geometric least-square means of all PK parameters were determined.

Results: Geometric means for Cmax, AUCinf and CL/F were respectively 4.3 ng/mL, 44.39 h·ng/mL and 3754.21 mL/min for ROSU alone, and 4.21 ng/mL, 45.89 h·ng/mL and 3631.30 mL/min for ROSU+PANTO. Ratio and 90% CI were 96.48% (83.67% - 114.24%), 100.20% (90.41% - 111.90%) and 99.58% (86.67% - 107.95%) for Cmax, AUCinf and CL/F, respectively. Geometric means for CLr were 245.82 mL/min (ROSU) and 232.24 mL/min (ROSU+PANTO). Ratio and 90% CI were 98.99% (87.73% - 101.73%). However, in one subject homozygous for variant alleles (521CC) associated with a loss of function in OATP1B1, an increase in plasma concentrations was observed following pantoprazole administration. Cmax and AUCinf were respectively 8.5 ng/mL and 83.83 h·ng/mL for ROSU alone, and 10.2 ng/mL and 90.33 h·ng/mL for ROSU+PANTO.

Conclusion: ROSU is an ABCG2 (BCRP) substrate for its elimination through the bile. However, this study showed that block of ABCG2 (BCRP) by pantoprazole does not alter rosuvastatin pharmacokinetics to a significant extent in healthy volunteers with functional activities of other membrane transporters involved in the disposition of the drug.

64. Placental Transfer of Formic Acid is Rapid and Decreases hCG Secretion

Hutson, JR[1,2], Lubetsky, A[1], Eichhorst, J[3], Hackmon, R[1], Koren, G[1,2], Kapur, BM[1,4]. [1]Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, Toronto, ON; [2]Insitute of Medical Sciences, University of Toronto, Toronto, ON; [3]Saskatchewan Disease Control Laboratory, Regina, SK; [4]Dept of Clinical Pathology, Sunnybrook Health Sciences Center, Toronto, ON

Corresponding Author: j.hutson@utoronto.ca

Conflict of Interest: None for all authors

Background: Formic acid has recently been detected in maternal blood and umbilical cord blood of infants born to alcohol abusing mothers. This toxic metabolite of methanol requires folate for detoxification. We hypothesize formic acid produced in the maternal circulation will transfer across the placenta and will be toxic to both the placenta and fetus.

Objectives: First, to determine whether formic acid transfers across the placenta and is toxic to the placenta. Second, to determine whether folate can decrease transplacental transfer of formic acid and mitigate toxicity.

Methods: Dual perfusion of a single placental lobule *ex vivo* was used to characterize the transfer of formic acid across the placenta. After a 1-hour control period, formic acid (2mM) was introduced into the maternal circulation with (n=4) or without folate (1uM) (n=4) and allowed to equilibrate for 3-hours.

Results: Formic acid transferred rapidly from the maternal to the fetal circulation and transfer was not altered with the addition of folate. Compared to the control period, there was a significant decrease in hCG secretion ($p=0.03$) after addition of formic acid. In contrast, there was no significant decrease when folate was present in the perfusate.

Conclusions: Formic acid rapidly transfers across the placenta and thus has the potential to be toxic to the developing fetus. Formic acid decreases hCG secretion in the placenta, which may alter steroidogenesis and differentiation of the cytotrophoblasts, and this can be mitigated by folate.

65. Maternal Fish Consumption and Mercury: Risk Perceptions and Therapeutic Monitoring

Jahedmotlagh Z[1,4], Schoeman K[1,2], Bend JR[1,2,3], Koren G[2,3,4,5]. Departments of Pathology[1], Physiology & Pharmacology[2] and Paediatrics[3], Schulich Medicine & Dentistry, University of Western Ontario, Ivey Chair in Molecular Toxicology[4], London, Ontario, Hospital for Sick Children[5], Toronto, Ontario
Corresponding Author: zjahedm@uwo.ca
Conflict of Interest: None declared

Background: While fish is rich in essential nutrients and women are encouraged to consume fish products, fish may contain methyl mercury which is an established neurotoxin to the fetus. Consequently there are high levels of anxiety among women of reproductive age regarding fish consumption.
Objectives: 1) To investigate what motivates women of reproductive age to avoid eating fish during their pregnancy and to understand their perceptions towards consuming fish; and 2) to pilot an intervention program in women of reproductive age to ensure mercury levels are below the LOAEL.
Methods: We surveyed 100 women of reproductive age who consulted the Motherisk program about fish consumption on their perceptions regarding fish consumption. Subsequently we implemented a therapeutic monitoring program for women who had hair mercury levels above 0.3 µg/g, the No Observable Effect Level (NOEL) for mercury for neurocognitive effects.
Results: The majority of women (90%) were aware of the potentially harmful effects of fish containing high levels of mercury. Most respondents were unable to describe specific toxic effects. When rating the level of anxiety from 0 (none) to maximal (10), the mean rate was 5, and 16 women were most worried.
A pilot on 5 women testing above the LOAEL of 0.3 mcg/g (mean 0.78±0.46), after diet modifications reducing fish consumption, levels decreased significantly (to 0.33±0.22) ($P<0.01$).
Conclusion: Women are very concerned about fish consumption and potential fetal risk. Therapeutic monitoring appears to be effective.

66. Transport of Lactic Acid by mct1 and mct4 in Cancer Cell Lines

Leung YH,[1,2] Belanger F, [2] Armstrong C,[1,2] Turgeon J [1,2]. [1]Faculty of Pharmacy, Université de Montréal, Montreal, QC, Canada; [2]CRCHUM, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, QC, Canada. Corresponding Author: jacques.turgeon@umontreal.ca
Conflict of Interest: No conflict of interest to report

Background and Objectives: A decrease in oxygen supply to mitochondria is associated with a reduction in ATP production from pyruvate and an increased formation of lactate. Accumulation of lactate, and secondly of lactic acid, is associated with mild to severe pathophysiological conditions including severe muscle pain. Two monocarboxylate transporters namely, MCT1 (SLC16A1) and MCT4 (SLC16A3), are involved in the transport of lactic acid in several cell types. The objective of our studies was to develop cell models to dissect activity of these two transporters.
Methods: Seven different breast cancer cell lines were cultured and harvested for RNA. RT-PCR analyses were performed to determine the relative mRNA expression of the MCT1 and MCT4. For uptake transport assay, cells were plated and incubated with [^{14}C]lactic acid at 37°C. The level of lactic acid incorporated was determined by using a liquid scintillation analyzer.
Results: RT-PCR results obtained allowed us to identify, out of the seven breast cancer cell lines, two cell lines with interesting characteristics. First, MDA-MB-231 expressed MCT4 at a much higher level than MCT1 while, on the other hand, SKBR3 expressed MCT1 at a greater level than MCT4. Experiments conducted to assess the functionality of the transporters in these cell lines confirmed the lactic acid uptake in both of those cell lines.
Conclusions: Different cancer cell lines can be used as in vitro models for the transport study of lactic acid. Experiments are currently underway to link MCT1 and MCT4 transport activity to drug toxicity.

67. Respiratory Depression Following Therapeutic Administration of Opioids in the Operating Room: An Opioid Pathway Pharmacogenetic Analysis

Madadi P[1], Sistonen J[2,3], Gladdy R[4], Silverman G[5], Carvalho JC[5], Ross CJD[2,3], Carleton BC[3,6], Hayden MR[2,3], Koren G[1]. [1]Clinical Pharmacology and

Toxicology, Hospital for Sick Children, Toronto, Canada; [2]Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada; [3]Child and Family Research Institute, University of British Columbia, Vancouver, Canada; [4]Dept. of Surgery, and [5]Dept. of Anesthesia and Pain Management, Mt. Sinai Hospital, Toronto, Canada; [6]Pharmaceutical Outcomes Programme, Children's and Women's Health Centre of British Columbia, Vancouver, Canada
Corresponding Author: parvaz.madadi@sickkids.ca
Conflict of Interest: None declared

Background: Systemic approaches are needed to understand how variations in the genes associated with opioid pharmacokinetics and pharmacodynamics can be used to predict clinical outcome. We present 2 cases of life threatening opioid-induced respiratory depression in the operating room. *Case One:* The patient had severe respiratory depression following 2 mg of subcutaneous morphine on top of intrathecal morphine administered for a Cesarean section. The patient had a history of near apnea with one dose of codeine/acetaminophen (30mg/500mg respectively), but tolerated hydromorphone. *Case Two:* Life threatening respiratory depression occurred following epidural morphine given at standard doses for surgical removal of tumor. Post-operatively, the patient needed only 0.6mg total of IV hydromorphone over 4 days for pain management.
Methods: Functional candidate polymorphisms in genes involved in opioid metabolism and action pathway (CYP2D6, UGT2B7, ABCB1, OPRM1, COMT) were genotyped by using SNaPshot® and TaqMan® Drug Metabolism Genotyping assays or by amplifying and re-sequencing the corresponding genomic regions.
Results: *Case One:* Genotype results revealed this patient had an increased propensity to generate active metabolites from both codeine (extensive CYP2D6 activity) and morphine (increased UGT2B7 activity) while having a functional μ-opioid receptor system. These active metabolites are not generated with hydromorphone. *Case Two:* Collectively, this patient appeared to have increased exposure and overall sensitivity to morphine and hydromorphone. Decreased ABCB1 efflux transporter activity, in combination with low COMT activity associated with increased sensitivity of the μ-opioid receptor system may have predisposed the patient to this adverse outcome.
Conclusions: An opioid pathway pharmacogenetic approach along with clinical history may provide insight into severe respiratory depressive events in patients who received therapeutic doses of opioids and may be useful information to mitigate future adverse events.

68. Rapid and Reversible Enhancement of Blood-brain Barrier (BBB) Permeability using Lysophosphatidic Acid (LPA)

On N, Miller DW. Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, Canada
Corresponding Author: ngocwpg@hotmail.com
Conflict of Interest: None declared

Background: The delivery of drugs to the CNS is limited due to the restrictive nature of the BBB. Transient modulation of BBB permeability is one method for enhancing drug delivery to the brain. One potential modulator of BBB permeability is LPA.
Objectives: Examine the use of the LPA, to transiently increase BBB permeability
Methods: LPA-induced alterations in brain microvessel permeability were examined in both cell culture and whole animal models. The permeability of fluorescein labeled dextran (FDX; MW3000) was examined using human brain microvessel endothelial cells, HBMEC, under control conditions and following exposure to LPA (0.1-10 μM). Effects of LPA on BBB permeability was examined in Balb/c mice using magnetic resonance imaging (MRI) and near infrared fluorescence imaging techniques.
Results: Exogenous LPA produced concentration-dependent increases in FDX permeability in HBMEC. Mice treated with LPA (1mg/kg) had significantly higher accumulation of Gadolinium contrast agent (Gd) compared to control mice in all regions of brain with the greatest increase observed in the posterior regions. The maximum enhancement of Gd occurred within 12 minutes following the administration of LPA. Re-establishment to normal barrier function was apparent within 20 minutes of LPA exposure. Examination of the permeability of a large near infrared fluorescent imaging agent, IRdye PEG, showed a qualitatively similar accumulation profile.
Conclusions: LPA produces a rapid and reversible increase in brain microvessel endothelia cell

permeability. These studies indicate that administration of LPA in combination with therapeutic agents may be an effective strategy to increase drug delivery to the brain.

69. Ocular Toxicity in Children Exposed in utero to Antimalarial Drugs: A Systematic Review of the Literature

Osadchy A, Ratnapalan T, Koren G. Motherisk Program, Division of Clinical Pharmacology & Toxicology, Hospital for Sick Children, Toronto, ON, Canada
Corresponding Author: alla.osadchy@sickkids.ca
Conflict of interest: The authors state no conflict of interest to declare

Background: There have been concerns regarding retinal toxicity in the offspring of women exposed to antimalarial drugs chloroquine (CQ) and hydroxycholoquine (HCQ) during pregnancy.
Objective: To systematically review the published evidence on safety of antimalarials during pregnancy with focus on ocular toxicity in the offspring.
Methods: Ovid MEDLINE(R), EMBASE and Cochrane Library databases were searched for randomized controlled trials (RCTs) and observational studies assessing visual function in the offspring of women exposed to antimalarials during pregnancy.
Results: 12 studies with a total of 588 exposed offspring met the inclusion criteria. Of 12 studies, 2 were RCTs and 10 were cohort studies 5 of which were lacking comparison group. Methods and time of visual assessment varied among studies. 5 studies reported no clinical visual abnormalities in all cases (n= 251). In a RCT on malaria prophylaxis, visual acuity in 251 infants exposed to CQ in utero did not differ from placebo group. Detailed ophthalmological examination was performed in 4 studies and normal results were reported in all children (n=59). Electrophysiological testing using electroretinogram was performed in 3 small cohorts of infants exposed to HCQ prenatally (n= 31) and were normal in all but six infants.
Conclusions: The current evidence from small and relatively low quality studies suggests no fetal ocular toxicity of antimalarials during pregnancy. The clinical significance of early electroretinogram anomalies reported in a small subset of infants remains to be established. Larger follow up studies are warranted to confirm low risk of ocular toxicity in children following antenatal exposure to antimalarial medications.

70. The Delirium Risk Evaluation and Assessment of Midazolam, EEG Recording and Sleep (DREAMERS) Study

Ovakim DH[1,2], Bosma KJ[2,3], Young GB[2,4], Sen M[2,3], Tirona R[1], Kim RB[1], Dresser GK[1]. [1]Division of Clinical Pharmacology, Department of Medicine, University of Western Ontario; [2]Critical Care Western, Division of Critical Care Medicine, Department of Medicine, University of Western Ontario; [3]Division of Respirology, Department of Medicine, University of Western Ontario; [4]Division of Clinical Neurological Sciences, University of Western Ontario
Corresponding Author: dovakim@uwo.ca
Conflict of Interest: None declared

Background: Critically ill patients require adequate sedation to tolerate the invasive interventions necessary for their care. Benzodiazepines are commonly used to achieve sedation in the intensive care unit (ICU) however they have been linked to the development of delirium. Midazolam, a benzodiazepine commonly used in ICU demonstrates significant interindividual pharmacokinetic (PK) variability in these patients.
Objectives: 1. To define the relationship between midazolam PK and electroencephalogram (EEG) in critically ill patients. 2. To clarify the effect of critical illness on midazolam pharmacokinetics. 3. To determine if impaired midazolam clearance is a risk factor for delirium.
Methods: Patients admitted to the ICU with sepsis and on a continuous infusion of midazolam were screened for study enrolment. Upon enrolment, continuous subhairline EEG was applied and daily blood samples were collected for plasma midazolam quantification. Clinical and laboratory parameters were followed and delirium onset was monitored using the Intensive Care Delirium Screening Checklist (ICDSC).
Results: Data is currently available for five patients. Patients on continuous midazolam infusions had maximal plasma midazolam concentrations ranging from 176 to 472 ng/ml (mean 336 ± 118). Corresponding EEG tracings demonstrated predominance of a delta wave pattern suggestive of deep sedation. Four out of 5 patients had ICDSC scores suggestive of delirium.

Comment: The midazolam concentrations observed suggest impaired clearance, and are higher than reported in other studies. Elevated midazolam levels, correlating with EEG recordings suggestive of oversedation may be a risk factor for the development of delirium. Further data is being analyzed and will be available for presentation.

71. Systematic Monitoring of Amiodarone Therapy Produces High Efficacy and Low Toxicity Rates

Pollak PT, Gillis AM, Wyse DG, Burland JB, Arburg B. University of Calgary, Calgary, AB, Canada. *Corresponding Author:* pollak@dal.ca
Conflict of Interest: None declared

Background: Amiodarone, although highly effective, is renowned for potential adverse effects limiting its use. In clinical trials of patients receiving arbitrary doses and minimal monitoring, 15-20% discontinue amiodarone in the first year.
Objectives: To report outcomes in patients taking amiodarone who are managed in a specialized Atrial Fibrillation (AF) Clinic with regular monitoring, individual serum drug concentration guided amiodarone dose titration, and focused education.
Methods: 60 patients, who were naïve to amiodarone when started in outpatient clinic, were followed for ≥ 12 months. Thyroid, liver and lung function were recorded at baseline, then monitored along with annual pulmonary metrics according to symptoms. Amiodarone dosing was adjusted using guidance from serum amiodarone concentrations.
Results: As a mixture of persistent and intermittent AF patients, 25% were in sinus rhythm at baseline (B). After 12 mo (E) of therapy, 0% discontinued amiodarone and 90% were in sinus without limiting adverse effects or clinically important changes in liver or thyroid function (ALT B=33 vs E=39 and FT4 B=14.9 vs. E=18.3). Thyroid supplementation was required in 15 pts (13 already on thyroxin at baseline) and later 4 pts developed transient hyperthyroidism that resolved while still on amiodarone. Some had transient neurologic symptoms that resolved after loading. No pulmonary toxicity was observed.
Conclusion: Systematic monitoring and dose adjustment based on serum amiodarone concentrations provides a high rate of therapeutic success. Thyroid issues were common, but no patient required discontinuation of amiodarone for toxicity.

72. Hair Cortisol Concentrations in Patients with Obstructive Sleep Apnea

Russell E[a], Koren G[a,b,c,d], Rieder MR[b,e], Rotenberg B[f], Ferguson KA[b], Uum SV[b]. [a]Department of Physiology and Pharmacology, University of Western Ontario, Canada; [b]Department of Medicine, Schulich School of Medicine and Dentistry, University of Western Ontario, Canada; [c]Ivey Chair in Molecular Toxicology, University of Western Ontario, Canada; [d]Department of Clinical Pharmacology/Toxicology, Hospital for Sick Children, Toronto, Ontario, Canada; [e]CIHR-GSK Chair in Pediatric Clinical Pharmacology, Children's Health Research Institute, London, Ontario, Canada; [f]Department of Otolarngology – Head and Neck Surgery, University of Western Ontario
Corresponding Author: erussel5@uwo.ca
Conflict of Interest: None declared

Background: Obstructive sleep apnea (OSA) is a common sleep disorder with serious cardiovascular and metabolic co-morbidities. OSA patients experience a repetitive collapse of the upper airway, resulting in intermittent episodes of hypoxemia. The adverse effects of OSA may be mediated by increased cortisol secretion. In this model, the frequent sudden arousals during sleep activate the hypothalamic-pituitary-adrenal axis resulting in increased nighttime cortisol secretion, a time when cortisol secretion is normally very low. Hair analysis is a non-invasive tool that can provide a retrospective, integral measure of cortisol production over several months.
Objectives: This study will assess if hair cortisol concentrations can be used as a biomarker of OSA severity. It is hypothesized that hair cortisol content correlates with the severity of OSA, and successful intervention with either CPAP or surgery will result in decreased cortisol concentrations.
Methods: Patients are recruited after undergoing a sleep study. Their hair cortisol concentrations are determined with an immunosorbent assay and compared with their apnea-hypopnea index (AHI), a score of the severity of a patient's OSA. A second, post-intervention sample will be collected from positively diagnosed patients undergoing an intervention.
Results: To date, 65 pre-intervention patients have been recruited, and many post-intervention hair

collections will occur in upcoming months. A preliminary analysis of 39 patients using a linear regression did not detect a correlation between cortisol concentration and AHI.
Conclusion and Plan: In March, 22 post-intervention samples will be collected, allowing for a preliminary assessment of if cortisol concentrations decrease with effective interventions for OSA.

73. Pharmacogenetics of Warfarin in Children

Shaw K[1,2,3], Amstutz U[1,2,3], Rassekh SR[4], Braun J[4], Ross C[3,5], Hayden MR[3,5], Carleton BC[1,2,3]. [1]Faculty of Medicine, Department of Paediatrics, UBC, Vancouver, Canada; [2]Pharmaceutical Outcomes Programme, BC Children's Hospital, Vancouver, Canada; [3]Child & Family Research Institute, Vancouver, Canada; [4]Division of Paediatric Hematology/Oncology/BMT, BC Children's Hospital, Vancouver, Canada; [5]Department of Medical Genetics, Faculty of Medicine, Centre for Molecular Medicine and Therapeutics, UBC, Vancouver, Canada
Corresponding Author: kshaw@popi.ubc.ca
Conflict of Interest: None declared

Background: Warfarin is a highly effective anticoagulant, but the large variation in dose requirements between patients makes dosing challenging. Inadequate warfarin dosing can cause serious adverse drug reactions (ADRs), such as blood clots or excessive bleeding. Polymorphisms in three genes (CYP2C9, VKORC1 and CYP4F2) have a significant effect on the required warfarin dose in adults. However, the validity of these findings have not been comprehensively assessed in children. Further paediatric studies are required to understand the predictive factors contributing to dose variation in children and prevent warfarin-induced ADRs.
Objectives: To determine the effect of genetic variation in CYP2C9, VKORC1 and CYP4F2 on warfarin response in children, as well as the importance of additional variation in genes involved in drug biotransformation and coagulation pathways that may be implicated in paediatric warfarin dosing.
Methods: We will collect a cohort of paediatric patients receiving warfarin therapy and test for associations of genetic variation in VKORC1, CYP2C9 and CYP4F2 with therapeutic dose, time to stable international normalized ration (INR), and warfarin-induced ADRs. We will also use univariate analysis to test for associations between therapeutic dose and additional genes studied.
Significance: The anticipated results of this study will provide insight into the genetic basis of warfarin dose requirements in children and the potential benefits of genetic testing in children prior to initiation of warfarin therapy. A paediatric-specific genetic dosing algorithm would allow early prediction of patients at risk for over- or underanticoagulation, and minimize the danger associated with warfarin therapy in this under-studied population.

74. Creation and Assessment of Adenovirus-Mediated Drug Transporter Model Expression System for the Prediction of Pharmacokinetic Profiles in Humans

Sugiyama D, Teft WA, Knauer M, DeGorter MK, Tirona RG, Kim RB. Division of Clinical Pharmacology, Department of Medicine, The University of Western Ontario
Corresponding Author: dsugiyam@uwo.ca
Conflict of Interest: None declared

Introduction: Predicting the in vivo role and effects of functional genetic polymorphisms to observed PK profile of drugs in humans is important for optimal drug therapy. Currently, IVIVE (in vitro to in vivo extraporation) algorithms are widely utilized for the prediction of drugs which are metabolized by CYP enzymes, but not transporters. Therefore, our goal is to create an in vitro transporter expression system capable of expressing multiple drug transporters simultaneously so that the cell-based system better reflects human organs such as the liver. Accordingly, we have cloned a number of hepatic bile acid and drug uptake transporters into an adenovirus-based expression construct and tested the efficiency of transporter expression in a number of cell lines.
Methods: Adenovirus constructs containing uptake transporters such as NTCP and members of the human OATPs were constructed and transport activity as well as cell viability assessed in HeLa, MDCKII, LLC-PK1 and Caco-2 cell lines.
Results: In terms of cell viability, LLC-PK1, Caco-2 and Hela were able to tolerate MOI (Mutiplicity of Infection) of 1000, but lower for MDCKII at MOI 300. Transport function at the same MOI was the greatest in HeLa cells. Interestingly, MDCKII cells exhibited higher transport activity compared to LLC-PK1 cells when expressing NTCP, but LLC-PK1

was better when expressing OATP2B1.
Conclusions: Our findings suggest a number of cell lines can be transduced to express hepatic transporters using the adenoviral expression system. Therefore our in vitro system has the potential serve as a physiologically relevant model for predicting in vivo PK.

75. Choroidal Antiangiogenic Effects of Lymphocyte-Derived Microparticles are Mediated through PEDF and Neurotrophin Receptor p75NTR Signalling Pathways

Tahiri H, Yang C, Gagnon C, Picard E, Chemtob S, Hardy P. Paediatrics & Pharmacology, University of Montreal, Montreal, QC, Canada
Corresponding Author: houda.tahiri@umontreal.ca
Conflict of Interest: None declared

Purpose: The importance of identifying VEGF-independent pathways in pathological angiogenesis is increasingly recognized as a result of emerging drug resistance to anti-VEGF therapies. Human T-Lymphocyte-Derived Microparticles (LMPs) significantly inhibit angiogenesis in several ocular neovascularization (NV). Both pigment epithelium-derived factor (PEDF) and the neurotrophins (NT) low-affinity p75NTR receptor have shown antiangiogenic effects. Our study is designed to determine how LMPs modulate the pro and antiangiogenic microenvironments in choroidal angiogenesis.
Methods: Antiangiogenic effects of LMPs were determined by using rat model of choroidal explants. LMPs were produced by treatment of human T-lymphocytes with actinomycin D. Cell viability (MTT assay), proliferation ([3H]-thymidine DNA incorporation), migration assays and apoptosis, were tested in cell lines. Choroidal expression of VEGF, PEDF, nerve growth factor (NGF) and p75NTR were demonstrated by Western blots and RT- PCR.
Results: Choroidal NV was suppressed by more than 50% after 72h of LMPs treatments. LMPs targeting acted on multiple cell types important for choroidal angiogenesis, such as vascular endothelial cells (inhibit HREC cell proliferation by 55%), and RPE cells (ARPE19). At a molecular level, LMPs regulated neurotrophins and their receptors expression both in vitro and in vivo. Inhibition of p75NTR abolished the antiangiogenic effect of LMPs.
Conclusions: LMPs are important candidate for antiangiogenic therapy. PEDF and neurotrophin induction by LMPs may be of therapeutic value in treating ocular neovascular diseases. Our data demonstrate that choroidal tissues have the capacity to synthesize neurotrophins, and that various stimulations can up-regulate gene and protein expression of neurotrophins.

76. Dehydroepiandrosterone Alters Retinol Levels and Expression of Retinol Related Proteins

Takitani K, Miyazaki H, Tamai H. Department of Pediatrics, Osaka Medical College. *Corresponding Author:* ped016@poh.osaka-med.ac.jp
Conflict of Interest: None declared

Background: Dehydroandrosterone (DHEA) and its sulfate DHEA-sulfate ester (DHEA-S) are the most abundant adrenal steroids in humans. However, the physiologic roles of DHEA and DHEAS have not been clearly defined. High levels of DHEA have been reported to be associated with decreased risk of cardiovascular disease and there has been speculation about their possible role in the aging process. In animal experiments, DHEA and DHEA-S have beneficial effects to obesity, diabetes, oncogenesis, atherosclerosis, and memory. Vitamin A is essential for vision, embryonic development reproduction, immunity, and growth. The affect of DHEA to retinol status has not been reported previously.
Objectives: In this study, we examined the retinol status in rats administered DHEA and investigated the expression of retinol related proteins including lecithin retinol acyltransferase (LRAT) and beta-carotene 15,15′ monooxygenase (BCM) genes, which are metabolic enzymes of retinol and beta-carotene respectively.
Methods: Wistar rats (four weeks, male) were assigned to two groups: a control group and a DHEA group fed the standard rat chow containing 0.4 % (wt/wt) DHEA, and fed for two weeks.
Results: Retinol levels of both plasma and liver in DHEA administered rats are decreased compared with controls. Hepatic BCM and LAT gene expression is significantly decreased in DHEA administered rats. Expression of both enzymes may affect circulatory retinol status. Conclusions: DHEA and DHEA-S are widespread as supplements for anti-aging. However, we should be aware that excess of DHEA intake might affect fat-soluble retinol status.

77. Behavioural Effects of Enhanced Expression of Equilibrative Nucleoside Transporter 1 in Mice

Sun C, Kost S, Xiong W, Parkinson F. Department of Pharmacology & Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, Canada.
Corresponding Author: chaosun86@hotmail.com
Conflict of Interest: None declared

Background: Adenosine is a neuromodulator that permeates cell membranes via nucleoside transporters. Mice with neuronal expression of human equilibrative transporter 1 (hENT1) have been generated (Parkinson et al., 2009 J. Neurochem. 109:562-572). Expression of hENT1 was associated with increased ataxic effects of ethanol and reduced stimulatory effects of caffeine, two drugs that act at least in part through adenosine signalling mechanisms.
Objectives: The present study examined the hypothesis that mice homozygous for the hENT1 transgene have significantly different behavioural responses to ethanol and caffeine compared with heterozygous mice.
Methods: To examine ethanol sensitivity, we tested loss of righting response (LORR) duration after injection (i.p.) of ethanol (3.6g/kg; 20% v/v in saline). To examine caffeine sensitivity, alterations in locomotor activity were monitored after injection of caffeine (25 mg/kg, i.p.).
Results: In behavioural assays, transgenic mice showed a greater response to ethanol and a reduced response to caffeine than wild type littermates, but no significant differences between heterozygous and homozygous transgenic mice were detected.
Conclusion: These data indicate that the increase in ENT1 function between wild type and heterozygous mice is greater than the increase in ENT1 function between heterozygous and homozygous transgenic mice. Therefore, homozygous mice do not offer a significant advantage over heterozygous mice for studies of ENT1 regulation of adenosine levels and adenosine dependent behaviours.

78. Cardiac Specific Over-expression of Membrane-associated Human Stem Cell Factor Promotes Epicardial Activation Post Myocardial Infarction

Xiang F-L [1], Lu X [3], Liu Y[1], Liu M [3], Feng Q [1,2,3]. Departments of [1] Physiology and Pharmacology, and [2] Medicine, University of Western Ontario, [3] Lawson Health Research Institute, London, Ontario, Canada
Corresponding Author: fxiang2@uwo.ca
Conflict of Interest: None declared

Background: Myocardial infarction (MI) is one of the leading causes of death worldwide. We recently demonstrated that the cardiac specific over-expression of membrane-associated human stem cell factor (MA-hSCF) improves cardiac function and survival post-MI. Epicardium derived cells (EPDCs), a population of cardiac stem cells which are essential for embryonic heart development, has recently been shown to be able to be activated and involved in cardiac repair post-MI.
Objective: The aim of the present study was to investigate the effects of cardiomyocyte-specific over-expression of MA-hSCF on epicardial activation post-MI. We hypothesized that cardiac specific over-expression of MA–hSCF promotes epicardial activation in mice post MI.
Methods and Results: Wild-type (WT) and the inducible cardiac-specific MA-hSCF transgenic (hSCF/ tTA) mice were subjected to MI. Activated EPDCs were increased in hSCF/tTA epicardium compared to WT mice ($P<0.05$) 3 days post MI as determined by Wt1 staining. E13.5 WT EPDCs were cultured and infected with Ad-hSCF or Ad-EGFP. Proliferation was significantly enhanced in Ad-hSCF infected EPDCs as determined by cell counting ($P<0.05$). Moreover, a trans-well system was employed to evaluate the migration of E13.5 $EGFP^+$ EPDCs. Neonatal WT cardiomyocytes were cultured and infected with Ad-hSCF or Ad-LacZ in the lower compartment of the trans-well. $EGFP^+$ EPDCs were seeded in the upper compartment. Twenty-four hours later, EGFP signal in the bottom well was significantly increased in the Ad-hSCF infected group compared to Ad-LacZ ($P<0.05$).
Conclusions: Cardiomyocyte-specific over-expression of MA-hSCF promotes the activation of EPDCs post-MI. Over-expression of MA-hSCF enhances the proliferation and migration of EPDCs.

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Wednesday, May 25, 2011

79. Chitosan Nanoparticulate System for the Delivery of siRNA Targeting Genome Caretakers for the Development of Potential Cancer Therapies

M. Alameh, D. De Jesus, M. Jean, M. Benhamadi, M.D. Buschmann, A. Merzouki. Institute of Biomedical Engineering, Department of Chemical Engineering, Ecole Polytechnique de Montreal, Montreal, Canada.

Purpose: The helicase superfamily of proteins plays major roles in numerous cellular processes including transcription, splicing, and DNA damage response. Many of these helicases have been demonstrated to be implicated in cancer pathogenesis. RecQL1, a DNA helicase that unwinds DNA in an ATP-dependent process, plays a major role in homologous recombination, maintenance of genomic integrity and DNA repair at damaged replication forks. RecQL1 is up-regulated in numerous tumor derived cell lines and its over expression is thought to prevent mitotic catastrophe. On the other hand DDX5 (p68) is an RNA helicase with an expression pattern correlating with tumor progression. DDX5 phosphorylation at Y593 promotes epithelial-mesenchymal transition via β-catenin nuclear translocation. Additionally, DDX5 double phosphorylation promotes resistance to TRAIL-induced apoptosis. Targeting these helicases using RNAi for the development of new therapeutics remains problematic due to a lack of efficient and safe delivery systems. Chitosan, a natural polymer of β-(1-4)-D-glucosamine and N-acetylglucosamine residues, is a promising delivery system successfully used both *in vitro* and *in vivo*. Here we demonstrate that specific chitosans can effectively deliver RNAi agent targeting the aforementioned helicases into numerous cell lines.
Methods: Chitosan-dsODN nanoparticles were characterised for size and charge density (ζ potential) using Dynamic Light Scattering (DLS) and Environmental Scanning Electron Microscopy (ESEM). Temporal stability of nanoparticles at two different pH and cargo protection against nucleases were assessed using gel retardation assays. Uptake efficiency was evaluated using cytometry and confocal microscopy. Transfection efficiency and gene silencing was measured using a qPCR TaqMan® assay.
Results: Chitosan-dsODN nanoparticles were found to be positively charged at ~ 20mV with an average size less than 100nm as demonstrated by DLS and ESEM respectively. Moreover, ESEM images indicated that nanoparticles were spherical, a shape promoting decreased cytotoxicity. Nanoparticle stability was demonstrated to persist for at least 20h at N:P ratios above 2 in slightly acidic pH. Moreover, increasing chitosan MW increased nanoparticle stability and their ability to protect dsODN from nucleases at supraphysiological concentrations (> 2U/μg dsODN). Furthermore, we determined that nanoparticles are able to attain high levels of uptake - 50 to 95% - in three different cells lines as demonstrated by flow cytometry. The high uptake efficiency correlated with high transfection efficiency as demonstrated by mRNA quantification level where knock down reached ~ 80% in LS174T without compromising cell viability.
Conclusion: Specific chitosans are efficient and non toxic siRNA delivery systems.

80. Inorganic Nanostructures for Enteric Delivery

D.C. Bassett[1], M.G. Cerruti[1,2], Y.L. Zhang[1] and J.E. Barralet[1,3]. [1]Faculty of Dentistry and [2]Department of Materials Engineering, McGill University, Montreal, Quebec, Canada; [3]Nanunanu, Birmingham, UK. david.bassett@mail.mcgill.ca

Purpose: In this study a novel preparation of highly porous nanostructured calcium phosphate microspheres that have previously been shown to sustain delivery of corticosteroid to the lung [1] are evaluated for their potential for enteric delivery of bioactives.
Methods: Microspheres were supplied by Nanunanu

Ltd. Morphological characterisation was performed using light and scanning electron microscopy. Specific surface area was assessed using nitrogen adsorption. Albumin was chosen for loading and release experiments. Protein loading was quantified using thermogravimetric analysis. Protein release was measured using the physiologically based extraction test (PBET) [2]. Loaded microspheres (10 mg) were placed in simulated gastric solution (10 ml) at 37 C and agitated by bubbling nitrogen. Aliquots (0.1 ml) were taken at 10 and 20 minutes at which point the PBET model was altered to the intestinal environment to simulate gastric emptying. Aliquots were taken every 2 minutes for 10 minutes during the intestinal release phase. The amount of albumin released was quantified using a BCA total protein assay. Protein degradation was evaluated using SDS-PAGE.

Results: The microspheres were uniformly spherical and had diameters in the range of 10 to 50 μm (Figure 1A-C). Microspheres were well dispersed both in aqueous (1A) and dry conditions (1B). At high magnification it was observed that the microspheres consisted of nanofibres 15-20 nm in diameter (1C &D) giving rise to a high specific surface area of 180 m^2g^{-1}. Up to 12 wt% albumin was successfully loaded onto the spheres. Upon immersion in the PBET model in gastric conditions, a small amount of albumin (11 % of total loaded) was released over the first 10 minutes of incubation; followed by a further 4 % over the following 10 minutes. Upon simulated gastric emptying, a significant release of albumin was recorded in the first 2 minutes (additional 26 %), and sustained release of approximately 7 % min^{-1} was recorded until 30 minutes at which point all of the loaded albumin had been released (Figure 2).

Conclusion: We have demonstrated successful loading and controlled release to a simulated intestinal environment of a model protein using a high surface area nanostructured inorganic microspherical carrier. This carrier appeared to be stable in the gastric environment, but rapidly released intact protein following gastric emptying into the intestinal environment. This release profile would appear to be ideal for targeted delivery to the upper part of the small intestine (duodenum / jejunum). These results are highly encouraging for the development of an enteric delivery system using these carriers which offer an alternative to conventional enteric coating systems.

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81. Carboxymethyl Starch: Chitosan Monolithic Matrices Containing Diamine Oxidase and Catalase for Intestinal Delayed Delivery

Carmen Calinescu[1], Bruno Mondovi[2], Rodolfo Federico[3], Pompilia Ispas-Szabo[1] and Mircea Alexandru Mateescu[1*]. [1]Department of Chemistry and Centre Pharmaqam, Université du Québec à Montréal, Montréal (Québec), Canada; [2]Department of Biochemical Sciences "Rossi-Fanelli", University of Rome "La Sapienza", Rome, Italy; [3]Department of Biology, University of Rome 3, Rome, Italy

Purpose: The diamine oxidase (DAO) catalyses the oxidative deamination of histamine and other biogenic amines, with the release of the corresponding aldehydes, hydrogen peroxide (H_2O_2) and ammonia (NH_3). Since DAO is a major catabolic enzyme for histamine in intestinal tract of humans, its lower mucosal level at the sites of inflammation would generate an accumulation of the released histamine, which may participate to the induction and enhancement of acute inflammatory responses. Another factor involved in the pathogenesis of intestinal inflammation is the oxidative stress. We are now proposing an oral enzymatic therapy based on the association of a vegetal DAO with catalase for the treatment of various colon diseases. The DAO would control the levels of histamine and would have some anti-oxidant effects. When associated, catalase will particularly eliminate the H_2O_2 by-product of DAO, preventing the local intestinal oxidative stress.

Methods: Oral enzyme formulations (monolithic tablets) based on Carboxymethyl high amylose starch (CMS) and Chitosan excipients and loaded with DAO vegetal extract (VDAO), catalase, or VDAO associated to catalase, as active principles, were obtained by direct compression of dry powders. The DAO enzymatic activity was determined with the peroxidase coupled reaction (specific for released H_2O_2) and with the L-Glutamate dehydrogenase coupled reaction (specific for released NH_3). The enzymatic activity of catalase was determined spectrophotometrically by

monitoring the H_2O_2 decrease during catalysis.
Results: The CMS:Chitosan (1:1) hydrophilic matrix afforded a good gastric protection to VDAO (30% loading) and to catalase (3.3% - 50% loading), when both were formulated separately, with 75% DAO activity and more than 80% catalase remaining activity after 60 min of incubation in simulated gastric fluid. A variable enzymatic activity of released DAO was found in simulated intestinal fluid, which was in function of the residence time of CMS:Chitosan tablet (30% VDAO) in the SGF. In the case of catalase formulated with CMS:Chitosan (10% loading), more than 50% of its initial enzymatic activity was found after 480 min in SIF, whereas, at higher loading, the percentage of released catalase activity was lower, probably due to protein-protein associations within the tablet. Concerning the bi-enzymatic VDAO:Catalase formulations based on CMS:Chitosan, the H_2O_2, the product of DAO activity, was decomposed by catalase which was liberated almost in the same time as DAO.
Conclusion: The anti-inflammatory DAO associated to catalase (clearing the pro-oxidant H_2O_2) and formulated with CMS and Chitosan excipients, could constitute a therapeutic approach in the treatment of intestinal diseases.

82. On-Demand, Targeted Drug Delivery using Magnetic Thermosensitive Nanocomposites

Scott Campbell, Elysia Jellema, and Todd Hoare. Department of Chemical Engineering, McMaster University, Hamilton, Ontario.

Purpose: Control of the site and rate of drug release is a persistent challenge in medicine, with no current devices offering precise, effective, user-defined, and site-specific release for a wide range of drugs. This research focuses on the development of drug-loaded, injectable, "smart" membrane-based microparticles and capsules that can both repeatedly deliver a drug "on-demand" and have site-specific functionality.
Methods: The membrane consists of poly(N-isopropylacrylamide) (PNIPAM)-based microgels and magnetic iron nanoparticles encased in an ethyl cellulose structural matrix. PNIPAM microgels are thermosensitive such that they reversibly decrease in size when the temperature of their environment exceeds their lower solution critical temperature (LCST). The magnetite nanoparticles can generate heat when placed in an oscillating magnetic field (OMF). When microgels and magnetite nanoparticles are combined in the same nanocomposite, heating of the nanoparticles induces a phase transition in the microgels, creating free volume in the microgel-templated pores and thus increased drug release. We are investigating two methods for fabricating such nanocomposites. First, we are entrapping microgels and magnetite nanoparticles inside an *in situ*-injectable hydrogel prepared by mixing hydrazide-functionalized PNIPAM and aldehyde-functionalized polydextran. Site-specificity can be achieved by injecting the composite at the desired site, where it quickly gels *in vivo*. Second, we are fabricating hollow sphere capsules by preparing water-in-ethanol-in-oil double emulsions, with ethyl cellulose (the solid support), microgels (the pore templating agent) and iron nanoparticles (the triggering agent) all contained within the central ethanol phase. The double emulsions will be manufactured using microfluidic channels that utilize multiple intersecting channels. This microfluidic technique allows for fabrication of the devices in a single synthetic step and allows for variations in particle size and membrane thickness through alterations in the flow rate ratios between intersecting microchannels.
Results: A wide range microgel-magnetite-hydrogel nanocomposites, with variations in the ratio of the two hydrogel polymeric components and microgel content, have been characterized for their swelling, degradation and drug release characteristics at 37°C and 43°C, as well as their drug release under the presence of an OMF. The optimal formulation for producing the double emulsions for capsule formation has also been determined using conventional double emulsification techniques and monitoring their stability. Results from the first few microfluidic device designs will be shown, along with the properties of the resulting nanocomposite materials.
Conclusion: The combination of thermosensitive polymers with magnetite nanoparticles is a powerful tool for drug delivery, permitting both site and temporal-specific control over drug release.

83. Therapeutic Lowering of Circulating ApoB in Atherosclerotic Mice by Intravenous Administration of Chitosan-siRNA Nanoparticles

D. DeJesus, M. Alameh, M. Jean, V. Lascau, M.D. Buschmann, A. Merzouki. Institute of Biomedical

Engineering, Department of Chemical Engineering, Ecole Polytechnique de Montreal, Montreal, Canada.

Purpose: Atherosclerosis is an inflammation mediated process that is initiated and promoted by low density lipoproteins (LDL) which carry cholesterol to the arteries. Each atherogenic LDL particle contains a unique and essential structural protein called apolipoprotein B (ApoB). Therefore, limiting the expression of ApoB will reduce the number of LDL particles formed, thus inhibiting the progression of atherosclerosis. We targeted the ApoB encoding messenger RNA(mRNA) using small interfering ribonucleic acids (siRNA) delivered using the natural polymer chitosan. This cationic, non-toxic, biocompatible polymer has been successfully used to deliver both plasmid DNA and siRNA both *in vitro* and *in vivo*, although never in the treatment of atherosclerosis.
Methods: Physicochemical characterization of chitosan-siRNA nanoparticles was performed using polyacrylamide gel retardation assays, Dynamic Light Scattering (DLS) and Environmental Scanning Electron Microscopy (ESEM). Transfection efficiency of these nanoparticles was assessed using flow cytometry and confocal microscopy in three cell lines. Gene inhibition levels were determined using quantitative polymerase chain reaction (qPCR) in a hepatocarcinoma cell line. The viability of transfected cells was assessed with alamar blue. We also studied the ability of the nanoparticles to deliver siRNA intravenously to the liver of mice presenting escalating levels of atherosclerosis. ApoB mRNA gene inhibition in the liver and jejunum was assessed through qPCR and confirmed using a mouse ApoB ELISA. The integrity of the liver was determined with a histological analysis of the organs and inflammation was measured using a mouse CRP ELISA.
Results: Chitosan-siRNA nanoparticles are stable spherical products with a diameter below 100nm and possess the ability to protect siRNA from circulating catalyzing enzymes. Furthermore, we determined that the nanoparticles are able to attain high levels of transfection in three cells lines and more than 50% of gene silencing in hepatic cell lines, without affecting cell viability. Finally, we determined the feasibility of intravenous delivery of chitosan-siRNA nanoparticles in atherosclerotic mice by achieving ApoB gene silencing higher than 50% and reducing circulating ApoB levels to those of healthy normal mice, without affecting the physiology and vital functions of the liver.
Conclusion: Physicochemical, *in vitro* and *in vivo* characterization of chitosan-siRNA nanoparticles revealed promising characteristics for siRNA delivery. High levels of ApoB mRNA gene inhibition were attained by intravenous administration and resulted in lower levels of circulating ApoB. Thus, siRNA delivery using specific chitosans is a promising approach for the treatment of atherosclerosis. Larger scale *in vivo* experiments are underway.

84. Effective and Safe Gene-based Delivery of GLP-1 using Chitosan/plasmid-DNA Therapeutic Nanocomplexes in an Animal Model of Type 2 Diabetes

M. Jean, M. Alameh, V. Daras, M. Lavertu, M. Thibault, M.D. Buschmann, A. Merzouki. Institute of Biomedical Engineering, Department of Chemical Engineering, Ecole Polytechnique de Montreal.

Purpose: To demonstrate effective and safe gene-based delivery of Glucagon-like peptide-1 (GLP-1) using chitosan/plasmid-DNA therapeutic nanocomplexes (TNCs) in a Zucker diabetic fatty (ZDF) animal model of type 2 diabetes. Glucagon-like peptide-1 (GLP-1) is an incretin hormone that regulates blood glucose level postprandially. It has been proposed that GLP-1 can be used in Type 2 diabetes mellitus treatment because of its insulinotropic action. Despite its remarkable advantages, GLP-1 suffers the disadvantage of an extremely short half life due to its degradation by the DPP-IV protease. One means of overcoming this drawback is GLP-1 gene delivery.
Methods: TNCs were prepared by mixing recombinant GLP-1 plasmid with chitosans of specific molecular weight (MW), degree of deacetylation (DDA) and ratio of chitosan amine to DNA phosphate (N:P ratio). Recombinant GLP-1 expression level and *in vivo* safety was systematically characterised in target tissues. Glucose metabolism in the diabetic ZDF rat model was assessed after intramuscular (IM), and subcutaneous (SC) administration of TNCs. Intraperitoneal glucose tolerance tests were performed to evaluate the efficacy and longevity of recombinant GLP-1 in ZDF rats treated with the TNCs.
Results: Animals injected with the TNC chitosan 92-10-5 showed GLP-1 plasma levels of about 5 fold higher than non-treated animals. The

insulinotropic effect of recombinant GLP-1 in treated animals was reflected by an increase in plasma insulin levels compared to negative controls. Intraperitoneal glucose tolerance tests revealed efficacious decrease of blood glucose to near-normal levels compared to controls for up to 24 days following treatment.
Conclusion: Therapeutic nanocomplexes composed of specific chitosans and GLP-1 expressing plasmid constructs demonstrated an impressive ability to harness the profound therapeutic potential of GLP-1 for treatment of Type 2 diabetes mellitus.

85. Nanoparticles with siRNA Targeted to Hsp70 mRNA Effectively Silence Hsp70 and Promote Apoptosis

Mirela Matokanovic[1], Karmela Barisic[1], Jelena Filipovic-Grcic[2], Anita Hafner[2], Dusica Maysinger[3]. [1]Department of Medical Biochemistry and Haematology and [2]Department of Pharmaceutical Technology, Faculty of Pharmacy and Biochemistry, University of Zagreb; [3]Department of Pharmacology and Therapeutics, McGill University.

Purpose: RNA interference (RNAi) is an endogenously present regulatory mechanism in eukaryote cells. It involves the destruction of messenger RNA (mRNA) upon interaction with homologous double stranded RNA (dsRNA), known as small interfering RNA (siRNA). Apoptosis resistance of tumor cells is associated with increased expression of inducible heat shock proteins (Hsp). SiRNAs targeted to Hsp gene family can be introduced into tumor cell in order to accomplish greater sensitivity of tumor cells to apoptosis.
Methods: In our study, aqueous solutions of chitosan, in the form of glutamate salt (molecular weight <200kDa; deacetylation 75-90%), sodium tripolyphosphate and siRNA were used for nanoparticles preparation (siRNA-NP) with N:P ratio of 100:1. The average particle size and size distribution (polydispersity index; PDI) was determined by photon correlation spectroscopy. The zeta potential was obtained by laser Doppler anemometry using a Zetasizer 3000 HS. Colorimetric MTT assay was performed to assess cell viability after 24h incubation with different volumes of siRNA-NPs. Quantitative RT-PCR was used for evaluation of siRNA(targeted against Hsp70)-NPs knockdown efficiency. Staurosporine was used as a model chemotherapeutic to study synergistic effect with Hsp70 gene silencing. The effect of siRNA-NPs on staurosporine induced apoptosis was measured with a quantitative immunoassay using mouse monoclonal antibodies directed against DNA (mono- and oligonucleosomes) and histones, that characterize apoptotic cell death.
Results: Mean particle size of prepared siRNA-NPs was in the nano-range (~170-220 nm) when incubated in serum free medium. Surface charge of prepared siRNA-NP was ~ +30 mV but decreased to +20 mV (± 2,2) when NPs were incubated in serum free medium, showing minor loss of chitosan positive charge in pH approaching pKa of chitosan. Cell viability was reduced to 80% (SD 5-10%) when different cell lines were incubated with 40 nM and 80 nM of siRNA entrapped into NPs. Hsp70 mRNA expression was significantly decreased after 24h incubation of cells with siRNA-NP in serum free medium. Expression levels of Hsp70 mRNA in different cell lines were approximately 56% of Hsp70 mRNA in negative control. Apoptosis was increased in the cells with posttranscriptionaly silenced Hsp70 gene.
Conclusion: Chitosan-based NP containing HSP70 SiRNA were successfully delivered into different cell lines. Hsp70 mRNA expression was significantly decreased (for 44%), and apoptosis was enhanced in cells exposed to siRNA-NP. These results indicate that siRNA70-NP could be effective in reducing the tumor growth in vivo.

86. Preparation and Characterization of Functionalized Carbon Nanotubes for Potential Biomedical Applications

Wei Shao, Satya Prakash. Biomedical Engineering Department, McGill University, Montreal, Quebec, Canada, Email: wei.shao2@mail.mcgill.ca

Introduction: Carbon nanotubes (CNTs) are novel nanomaterials made from carbon and belong to family of fullerene. Functionalized CNTs are water-soluble and biocompatible, and therefore suitable for biomedical application. CNTs, as newly emerging biomedical material, have brought attentions for biomedical applications. Needle like CNT exhibits unique properties, such as high surface area, nanosized stability. More importantly, CNTS show very good cell penetrating property and can easily penetrate all sorts of cells, including mammalian, yeast and bacteria cells. One of the major problems

encountered in cancer therapies is off-target tissue toxicity. CNTs, with high surface area to volume ratio and excellent cell penetration property, are excellent carriers for efficient loading of cancer targeting molecules, imaging agents and cancer therapeutics. In this study, water soluble CNTs were prepared with polymers that contain function groups for further conjugation of drug or other therapeutics. The functionalized CNTs were evaluated for potential biomedical applications.

Methods: Single walled carbon nanotubes (SWNTs) were functionalized with biocompatible amphiphilic block polymers that containing functional end groups for potential drug conjugation. The formation of SWNT-polymer conjugates was characterized with TEM and UV-NIR absorptions. Cell penetration property of SWNT-polymer was evaluated by fluorescent-labeled SWNT-polymers. The physical and chemical stability SWNT-polymer-drug conjugation were evaluated in water, culture media and plasma conditions. Cytotoxicity of SWNT-polymer conjugate was investigated in cell lines using MTS assay and apoptosis assay.

Results: a soluble CNT-polymer based drug carrier system was generated and evaluated for potential cancer drug delivery. TEM result confirmed the attachment of polymers to SWNT sidewall and formation of brush-like structure. The polymer functionalized SWNT was stable in water, culture medium for tested period of three months. Incubation of fluorescence-labeled SWNT-polymers with cell line MCF-7 showed that polymer functionalized SWNT had strong cell penetrating capability. MTS assay showed that incubation of increased amounts of polymer-SWNT (0.2 – 1 μg/ml) with MCF-7 cells for up to 21 hrs was not toxic compared with non-treated cells. This result was further confirmed by cell apoptosis assay using Annexin V.

Conclusions: The evaluated polymer – CNTs conjugates has superior aqueous stability, strong cell penetrating capability, good biocompatibility and potential for efficient drug loading, which makes it a promising drug delivery platform for further development.

87. Towards Delineating the Cellular Uptake of Three-Dimensional DNA Nanostructures

Johans Fakhoury, Christopher McLaughlin, Hanadi Sleiman. Faculty of Science, Chemistry Department, McGill University, Montreal, Quebec, Canada

Purpose: Traditional chemotherapy has been one of the major weapons to target cancer cells. Unfortunately, the majority of clinically approved chemotherapeutic anti-cancer agents target cell growth and are not specific to cancer cells. Most of these agents target fast growing tissues and have substantial detrimental side-effects.

Nanoparticles offer several advantages over traditional chemotherapeutics: small particle size, narrow size distribution, modularity of surface features for targeted drug localization, and protective isolation of drug molecules for enhanced molecule stability.

We have shown that unlike previously used nanoparticles, we are able to modulate the length, diameter, and shape of DNA nanostructures. Additionally, we have demonstrated that these DNA nanostructures possess a robust encapsulating ability of various cargo materials.

We hypothesize that by functionalizing the surface of DNA nanostructures with folate molecules we will construct highly specific cancer targeting vehicles. Folate receptors (FR) have been shown to be highly enriched on the surface of many types of cancer cells, including cancer cells from lung, brain, breast, ovarian, and uterine carcinomas, while a decreased folate receptor expression has been observed in normal and non-cancerous cells.

Methods: We propose to simultaneously conjugate DNA nanostructures to folate for efficient FR targeting, and to a fluorescent dye molecule for reproducible tracking and observation. We will determine their stability over various in vitro conditions and their interaction with human cancer cells. Once the uptake of DNA nanostructure-folate molecules is characterized, we propose to encapsulate traditional cancer therapeutics such as platinum-based agents and antisense oligonucleotides and study their efficiency and specificity towards cancer cells.

Results: We have constructed DNA nanosturctures with detectable fluorescent molecules attached to the surface. We have also shown that these DNA structures are highly stable in solution and resistant to denaturing conditions.

Conclusion: We predict that by generating highly specific anti-cancer folate-conjugated DNA nanostructures we will be able to increase the specificity of pre-established cancer therapeutics without increasing their cytotoxicities. Additionally, we will be able to overcome drug resistance generated by the usage of high doses of conventional

therapeutics.

88. Excess Polycation Mediates Efficient Chitosan-based Gene Transfer by Promoting Lysosomal Release of the Polyplexes

Marc Thibault, Mélina Astolfi, Nicolas Tran-Khanh, Marc Lavertu, Vincent Darras, Abderrazzak Merzouki, Michael D. Buschmann. Department of Chemical Engineering, École Polytechnique, Montréal, Québec, Canada.

Purpose: The optimal ratio of the polycation's amine to DNA phosphate group (N:P) for efficient polymer-based transfection always employs excess polycation versus DNA. Most of the excess polycation remains free in solution, unassociated with the polyplexes, but is essential for efficient transfection. The mechanism by which excess polycation increases transfection efficiency is not yet identified. We hypothesised that excess chitosan facilitates intracellular lysosomal escape of the polyplexes.
Methods: We highlight here the essential role of excess chitosan by rescuing poorly transfecting low N:P ratio polyplexes, by adding free chitosan before or after polyplex addition to cells. We examined polyplex uptake, the kinetics of rescue, intracellular trafficking, and the effects of lysosomotropic agent chloroquine. Kinetics of the lysosomal transit was analysed by confocal live-cell imaged-based quantification of colocalisation between fluorescently-labeled polyplexes and lysosomes.
Results: We found the facilitating role of excess chitosan to be downstream of cellular uptake and involving endo-lysosomal vesicular processing. Live cell confocal quantification of intracellular trafficking revealed prolonged colocalisation of low N:P polyplexes within lysosomes, compared to shorter residence times for both rescued or N:P 5 samples, followed by observation of free pDNA in the cytosol.
Conclusion: These data demonstrate that excess polycation mediates enhanced transfection efficiency by promoting the release of polyplexes from the endo-lysosomal vesicles, revealing a critical intracellular barrier overcome by excess polycation and suggesting possible avenues for further optimisation of polymer-based gene delivery.

89. Traceable Micellar Nanomedicine for Cancer-Targeted Co-delivery of siRNA and Doxorubicin for Multidrug Resistantce Reversal

Xiao-Bing Xiong and Afsaneh Lavasanifar. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Purpose: The purpose of this study is to develop traceable micellar nanomedicine for effective treatment of multidrug resistant cancers by co-delivery of doxorubicin (DOX) and mdr1-siRNA and for tracking the nano-delivery in vitro and in vivo by molecular imaging.
Methods: Multifunctional micellar nanomedicine was constructed from poly(ethylene oxide)-*block*-poly(caprolactone) (PEO-*b*-PCL) based virus-mimetic micelle, which has a versatile core for complexing siRNA, DOX and imaging probes, and a virus-mimetic shell conferred by the dual functional moieties, i.e. integrin αvβ3-targeted RGD for cell-specific recognition and cell penetration peptide TAT for efficient cell membrane translocation, respectively.
Results: The multifunctional micelles were successfully prepared with a particle size of ~ 100 nm. The formation of mixed micelles was confirmd by two-color flow cytometry analysis and AFM. The pH-triggered DOX release from the micelle was demonstrated. RGD and TAT modified micelles (RGD/TAT-micelles) showed significantly higher cellular uptake of siRNA and DOX compared to RGD or TAT-modified micelles. Confocal microscopy revealed efficient endosome escape of DOX and siRNA for RGD/TAT-micelles. Multifunctional micelles with complexed mdr1 siRNA and DOX significantly increased DOX accumulation in P-gp overexpressing MDA435/LCC6 resistant cells and led to DOX resistance reversal. Optical in vivo imaging showed that RGD-micelles and their payloads selectively accumulated in tumor tissue.
Conclusions: The results of this study demonstrated a tremendous potential of this multifunctional micellar nanomedicine for cancer targeting, efficient delivery of anticancer and siRNA delivery to its cellular and molecular targets, and meanwhile tracking the in vivo delivery by molecular imaging.

NHPRS Posters - Day 1

Wednesday, May 25, 2011

Poster Session 1

90. Testing the Effects of Electrophilic Plant Secondary Metabolites in Mammalian Cells by RNA-SEQ (NHPRS-P1-01)

David J. Konkin[1,2] and Jon E. Page[1,2] . [1]Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. [2]National Research Council–Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada.
Email: David.Konkin@nrc.ca

Plants produce an array of secondary metabolites (phytochemicals) that function as defence against herbivores and pathogens, or attractants for pollinators. Recently interest has risen on the potential for plant secondary metabolites to prevent or delay chronic diseases by moderating redox stress and inflammation. Indeed, plant secondary metabolites that induce the expression of cytoprotective genes by stabilizing the transcription factor Nrf2 display encouraging results in animal models and preliminary studies. However, major questions remain with regard to this approach for disease prevention. What plant secondary metabolites are potent enough to have physiological relevance at concentrations achievable in vivo? What are the off-target effects of these compounds? How do these compounds affect global gene expression at the concentrations required for protective action? How do these compounds affect the expression of phase I and II drug metabolizing enzymes? We have isolated several phytochemicals, including polyacetylenes and phthalides from the plants Oplopanax horridus (devil's club) and Ligusticum porteri (ósha), respectively, that induce the expression of the protective enzyme quinone reductase, a well characterized target of Nrf2, in mouse hepatoma cells. These compounds all possess electrophilic moieties and thus have a high potential for modifying proteins with nucleophilic residues and off-target effects. We used an RNA-sequencing approach in order to gain an understanding of the global changes in transcription that occur in cells exposed to these compounds (at concentrations required to induce quinone reductase expression) and provide insight regarding the signalling pathways that are affected by these compounds.

91. Prevalence and Predictors of Supplement Use in a Urology Population (NHPRS-P1-02)

Jennifer Locke[1], Karen Hersey[1], David Margel[1], Emma Tomlinson Guns[2], and Neil Fleshner[1]. [1]Urology Program, Princess Margaret Hospital-University Health Network, Toronto, Ontario, [2]Vancouver Prostate Centre, Vancouver, British Columbia. Email: Jennifer.locke@utoronto.ca

Introduction: Aside from family history and ethnicity, diet has long been implicated as having a role in prostate cancer. In this study we aim to investigate the current prevalence and predictors for use of several supplements by men with or at risk for developing prostate cancer in a urology population.
Methods: 312 men visiting the Princess Margaret Hospital ambulatory urology clinic in Toronto were enrolled in this University Health Network Research Ethics Board approved questionnaire-based study investigating the use, past use and reasons for use of several supplements.
Results: We observed that 89% of these men are currently using one or more forms of these supplements. Many are taking vitamin D (57%), calcium (31%) and vitamin C (35%). Significantly fewer are taking green tea (2%), ginseng (3%) and saw palmetto (8%). Most men attributed their reasons for current use of these supplements to urologist advice and family member advice as compared to other reasons investigated. Interestingly, some men discontinued the use of vitamin E (16%), selenium (11%) and saw palmetto (12%) and attribute this discontinuation to urologist advice and periodical readings as compared to other reasons investigated. Although predictors for current and past use varied based on supplement type, education level emerged as significant

predictor on both univariate and multivariate analyses for many supplements used.
Conclusion: Many men continue to use supplements for varying reasons. Information presented here may contribute to improved education of these men surrounding the use of these supplements in the prevention and treatment of prostate cancer.

92. The Flower and The ripe Fruit of Tunisian Pomegranate (*Punica granatum*) Stimulate Glucose Transport in C2C12 Muscle Cells Through the AKT and AMPK Pathways (NHPRS-P1-03)

Amine Ben Abdennebi– [1], Lina Musallam [1], Diane Vallerand [1], Jalila Bakir [2], Mohamed Mars [2], Pierre S. Haddad[1]. 1) Laboratoire de produits de santé naturels et de maladies métaboliques, Département de pharmacologie, Faculté de médecine, Université de Montréal. 2) Faculté des Sciences de Gabès, Campus Universitaire, Tunisie, Tunisie.

Diabetes is a major public health problem worldwide with astounding human and economic consequences. The seed and the flower of Pomegranate (*Punica granatum*), a native plant of Central Asia and the Mediterranean regions, exhibited a hypoglycaemic effect in *in vivo* studies. However, the underlying mechanisms have not yet been elucidated. The aim of this project was to evaluate the effect of the flower and the fruit (at 3 maturation stages) of Pomegranate on glucose transport in skeletal muscle cells and to determine the molecular mechanisms involved in this effect. To accomplish this, we chose three varieties of Pomegranate cultivated in Tunisia (Gabsi [Gab], Garsi [Gar] and Espagnoule [Esp]) which have been shown to be highly exported, highly consumed in that area or to possess high antioxidant activity, respectively. Differentiated C2C12 cells were treated for 18h with 80% ethanolic extract of the flowers and fruits (at 2, 4, and 6 months) of each variety. Our results show that the Gabsi variety of Pomegranate significantly enhances glucose uptake by 10-20% as compared to the control (DMSO), without any toxicity. This effect is more pronounced in the ripe fruit (6 months) than in the flower. In parallel, the flower and the ripe fruit stimulate both the insulin-dependent pathway (Akt) and the insulin-independent pathway (AMPK), albeit the latter more potently. Hence, these results suggest that regulation of glucose transport in skeletal muscle is one of the components involved in the anti-diabetic effect of Tunisian pomegranate. Funded by CIHR and the Government of the Tunisia.

93. Latitudinal Variation of Phytochemical Compounds in Labrador Tea, *Rhododendron greonlandicum* and Pitcher Plant, *Sarracenia purpurea* (NHPRS-P1-04)

Michael Rapinski[1] Rui Lilin[2] Asim Muhammad[2], Ammar Saleem[2] John Arnason[2], Alain Cuerrier[3]. [1]Institut de Recherche en Biologie Végétale, Montréal, Québec, Canada; [2]Department of Biology, University of Ottawa, Ottawa, Ontario, Canada; [3]Jardin Botanique de Montréal, Montréal, Québec, Canada. Email: michael.rapinski@umontreal.ca

Rhododendron groenlandicum and *Sarracenia pupurea* are used as traditional medicines by the Cree Nations of Eeyou Istchii to treat diabetes related symptoms and have exhibited antidiabetic activity in 3T3 cell adipogenesis and C2C12 cell glucose transport respectively. Through ethnobotanical surveys, it has been mentioned that the plant's medicinal potential augments in northern communities. While there are few studies focused on the effect of a latitudinal gradient on a plant's biological activity, there is evidence that phenolic compounds are produced in greater quantities as latitude increases to protect the plants from photoinhibition. With longer daytime periods occurring at northern latitudes over the growing season, we hypothesized that northern populations of S. Purpurea and R. Groenlandicum will have higher concentrations of phytochemicals and a stronger biological activity. Accessions from the surroundings of five Cree communities in the James Bay area were collected on a north-south gradient and extracted in 80% EtOH. Polyphenols were identified and quantified by applying a novel analytical method using RP-HPLC-DAD-ELSD. Though not linearly related to latitude and day length, phytochemical results indicate a geographical variation in polyphenol concentrations and distinct chemotypes between communities. Antidiabetic bioassays are currently being used to analyze these samples in vitro in order to assess the biological significance of this phytochemical variation.

94. Screening and Bioassay-Guided Fractionation of Anti-Diabetic / Obesity and Neuroprotective Components from Dietary Chinese Herbs (NHPRS-P1-05)

Kaibin Zhang[1,2], Xiaoli Sun[1,2,3], Xiuhong Ji[1], Yanwen Wang[1], Jeffrey Zidichouski[1], Zhimin Wang[2,*], and Junzeng Zhang[1,*]. 1Institute for Nutrisciences and Health, National Research Council Canada, Charlottetown, PE, Canada. 2 Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China. 3 School of Traditional Chinese Medicine, Capital Medical University, Beijing, China. Email: junzeng.zhang@nrc.gc.ca; zhmw123@263.net

In China, a number of herbs are regulated both as medicine and food, called dietary herbs. These dietary herbs could be beneficial for prevention or treatment of chronic health problems such as obesity/diabetes and neurodegenerative diseases. In order to investigate the bioactive components of these herbs, we first prepared 63 water or aqueous EtOH extracts from selected dietary Chinese herbs and tested their ability to inhibit the activity of several enzymes or act on other targets. We report here the preliminary results obtained from a set of initial screenings. INH-OS-50 and INH-OS-94 at 0.37 mg/mL inhibited acetylcholinesterase (AChE) activity by 33.1% and 34.6%, respectively (the positive control eserine at 0.148 mg/mL totally inhibited the enzyme activity); INH-OS-42 and INH-OS-61 at 75 μg/mL inhibited human islet amyloid polypeptide (hIAPP) aggregation by 83.52% and 77.61%, respectively (70.82% for the positive control, curcumin at 25 μg/mL); INH-OS-38 and INH-OS-103 at 0.11 mg/mL inhibited beta amyloid 1-42 aggregation by 78.50% and 63.18%, respectively (47.82% for the positive control, curcumin at 0.43 μg/mL); INH-OS-38, INH-OS-59 and INH-OS-60 at 0.63 mg/mL inhibited α-glucosidase activity by >90% (comparable or higher than that of positive control, acarbose at 0.25 mg/mL). We also tested the lipase inhibitory activity of the 22 extracts, INH-OS-107 at 8.75 μg/mL inhibited pancreatic lipase activity by 60.63% (95.45% for the positive control, orlistat at 8.75 μg/mL). Further bioassay-guided fractionation, purification and structure characterization are presently underway.

Poster Session 2

95. Assessment of the Anxiolytic Properties of *Rhodiola rosea* L. in Mice: Comparison of Acute and Chronic Oral Administration (NHPRS-P2-01)

Fida Ahmed[1,2], Carolina Cieniak[1,2], Chris Cayer[3], Steffany A.L. Bennett[2], John T. Arnason[1]. [1]Centre for Advanced Research in Environmental Genomics (CAREG), Department of Biology, University of Ottawa. [2]Neural Regeneration Laboratory and Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology, and Immunology, University of Ottawa. [3]Department of Psychology, University of Ottawa.

Rhodiola rosea L., also known as roseroot, goldenroot or Arctic root has a well-established tradition of use for alleviating mental and nervous system disorders, particularly in circumpolar Eurasia. In Canada, *R. rosea* is used by the Inuit to promote mental well-being. Of particular interest is its ethnobotanical use in relieving anxiety. Previously, our group has demonstrated that Canadian *R. rosea* has significant anxiolytic activity after acute oral administration in rats. In this study, we assessed the effects of both acute and chronic administration of *R. rosea* in mice on several measures of anxiety. *R. rosea* extract was administered orally to two-month old male mice daily using diluted condensed milk as a vehicle for a period of one week (acute) and eight weeks (chronic) at 100 mg/kg body weight. The mice were then subjected to a series of behavioural tests including the elevated-plus maze, the light-dark box, the open-field and the social interaction tests to assess effects on anxiety. The differences in anxiolytic activity of *R. rosea* acute treatment between rats and mice were explored. Surprisingly, we observed that chronic administration does not inhibit behavioural indices of anxiety, suggesting tolerance over time. These results suggest that the period of treatment may greatly influence the anxiolytic effects of *R. rosea*.

96. Systematic Review of the Efficacy of Bioidentical Hormones in Reducing Symptoms of Menopause. Part 1. Progesterone and Relief of Menopause-Related Vasomotor Symptoms. (NHPRS-P2-02)

Tannis M. Jurgens[1], Anne Marie Whelan[1,2] , Melanie Trinacty[1]. [1]College of Pharmacy, [2]Department of Family Medicine, Dalhousie University, Halifax NS Canada. Email: tannis.jurgens@dal.ca

Objectives*:* Bioidentical hormones (BHs) are widely used, yet we don't have a clear definition of what they are and what the evidence of their efficacy is. The objectives of this project were to 1) clearly define "bioidentical hormone" and 2) to conduct a systematic review (SR) of the evidence of efficacy of bioidentical progesterone in the relief of menopause-related vasomotor symptoms.
Methods*:* A systematic search of the literature and internet was conducted to identify definitions of BHs. Definitions were examined for similarities and differences and a working definition was created. A systematic search of databases and literature including: Pubmed, Embase, IPA, CINAHL, Cochrane and the Intern J Pharm Comp, was conducted to identify randomized controlled trials (RCTs) of progesterone in the management of menopause-related vasomotor symptoms.
Results*:* Analysis of 62 definitions of BHs resulted in the creation of the working definition: "Bioidentical hormones are chemical substances that are identical in molecular structure to human hormones." Database searching resulted in 359 unique records, 35 of which passed the screening for relevance. Four of the 35 articles met criteria for inclusion in the SR. Critical appraisal identified significant shortcomings in trial design. Three of 4 RCTs did not report a significant difference with the use of progesterone for menopause-related vasomotor symptoms as compared to placebo.
Conclusions*:* The working definition of BHs should facilitate a common understanding of the term among the scientific and public communities. Current evidence from the reviewed RCTs does not support the use of progesterone for menopause-related vasomotor symptoms. *Funding:* This project was supported by the Dalhousie Pharmacy Endowment Fund.

97. AChE Inhibition and Neuroprotective Activity of Novel Eremophilanes from *Senecio jacobaea* in SH-SY5Y Cells (NHPRS-P2-03)

Xiuhong Ji[1], Fei Yin[1,2], Jianhui Liu[1,2], Lucas Rivers[1], Zosia Zielinski, Charmaine Noonman[1], Jeffrey Zidichouski[1], Yanwen Wang[1], Christopher Kirby[3], and Junzeng Zhang[1,*]. [1]Institute for Nutrisciences and Health, National Research Council Canada, 550 University Avenue, Charlottetown, Canada. [2]Research Center of Medicinal Chemistry and Chemical Biology, Chongqing Technology and Business University, Chongqing, PR China. [3]Crops and Livestock Research Centre, Agriculture and Agri-Food Canada, Charlottetown, PEI, Canada.
Email: junzeng.zhang@nrc.gc.ca

Acetylcholinesterase (AChE) inhibitors are currently used as the standard care and symptom modification drugs for Alzheimer's disease (AD). In our effort of searching natural AChE inhibitor from Atlantic Canada plant extracts, we reported previously two compounds 6□,8□-dimethoxy-1-oxoeremophil-9(10)-en-8□,12-olide (**1**) and 6□-angeloyloxy-8□-hydroxy-1-oxoeremophil-9(10)-en-8□,12-olide (**2**) isolated from the root of a common plant Tansy Rugwort *Senecio jacobaea* with potent AChE inhibitory activity. Here we present another novel eremophilane analog 6□-Angeloyloxy-10□-hydroxy-1-oxoeremophil-8(9)-en-8,12-olide (**3**) with similar AChE inhibition potency. The AChE inhibitory activities of these 3 compounds were also verified in SH-SY5Y cells, and the IC_{50} determined are 17.8, 20.0, and 5.5 □M for compounds **1**, **2**, and **3** respectively. In order to understand other possible benefits of these compounds for neuroprotection, their effects on cell viability with the presence of glutamate, Abeta 1-42, and H_2O_2 were evaluated and all showed neuroprotective activities in a dose-dependent manner. These eremophilane compounds represent a new class of AChE inhibitors and **2** and **3** are also novel compounds. The discovery may lead to development of new AChE inhibitors for managing mild AD symptoms.

Poster Presentations
Day 2
Thursday, May 26, 2011

CSPS Posters - Day 2

Thursday, May 26, 2011

Biomedical Sciences

98. Differential Effects of Clinically Important Glucocorticoids on Active Vitamin D_3 Metabolism in Mouse Liver: Involvement of Cytochrome P450 3A Isoforms

Subrata Deb, Mitali Pandey, Hans Adomat and Emma Tomlinson Guns. The Vancouver Prostate Centre, Vancouver, BC, Canada.

Purpose: The chemopreventive and therapeutic effects of vitamin D_3 are exerted through 1α,25-$(OH)_2D_3$, the dihydroxy metabolite of vitamin D_3. Hepatic inactivation of 1α,25-$(OH)_2D_3$ by cytochrome P450 (CYP) enzymes can be an important determinant of it's circulating serum and tissue levels. Vitamin D-deficiency like symptoms were observed in patients receiving dexamethasone as premedication before taxane-based chemotherapy. The purpose of the present study was to compare the effects of clinically important glucocorticoids, such as dexamethasone and prednisone, on inactivation of 1α,25-$(OH)_2D_3$ in mouse liver and to identify the CYP enzyme(s) involved in the metabolism of 1α,25-$(OH)_2D_3$.
Methods: Adult male CD-1 mice were treated with either vehicle (50% ethanol), dexamethasone (80 mg/kg/day) or prednisone (80 mg/kg/day) for three consecutive days by intraperitoneal injection. Livers were quickly excised and were used for preparation of microsomes by differential ultracentrifugation. A liquid chromatography-mass spectrometry method was developed to analyze metabolite formation and substrate depletion following *in vitro* biotransformation of 1α,25-$(OH)_2D_3$. Reaction phenotyping was carried out using a panel of chemical inhibitors for CYP1A, CYP2B, CYP2C, CYP3A, CYP2D and CYP2E enzymes. Incubations were also carried out using human hepatic microsomes for comparative purposes.
Results: The hydroxy metabolite formation pattern of 1α,25-$(OH)_2D_3$ was similar in vehicle- or prednisone-treated groups, whereas treatment with dexamethasone led to emergence of additional metabolites. Enzyme kinetic parameters were significantly different in dexamethasone-treated mice with substantial increase in formation of 1α,24*R*,25-$(OH)_3D_3$ and 1α,23*S*,25-$(OH)_3D_3$ compared to vehicle- or prednisone-treated mice. Interestingly, metabolite formation profile in dexamethasone-treated mice was comparable to that of human liver microsomes. Substrate depletion analysis suggest that dexamethasone is ~70% more efficient than vehicle- or prednisone-treated mice in inactivating 1α,25-$(OH)_2D_3$ (1μM). Ketoconazole, a potent mouse CYP3A inhibitor, blocked 1α,25-$(OH)_2D_3$ metabolism in hepatic microsomes from vehicle-, prednisone- or dexamethasone-treated mice by 80-100%. Similarly, CYP3A protein levels and triazolam hydroxylation, a mouse CYP3A marker assay, were significantly increased in dexamethasone-treated, but not in vehicle- or prednisone-treated microsomal samples.
Conclusion: Dexamethasone augments CYP3A-mediated hepatic inactivation of 1α,25-$(OH)_2D_3$, the active metabolite of vitamin D_3, in mice, whereas prednisone has little or no effect on inactivation of 1α,25-$(OH)_2D_3$.
Acknowledgements: This work was supported by funding from Motorcycle Ride for Dad and Sanofi Aventis to EG. A Grant-in-Aid financial support was provided to SD by Prostate Cancer Foundation of BC, Canada.

99. Screening of CD36 Synthetic Ligands by Surface Plasmon Resonance (SPR)

Patrick Lambert-Lanteigne1, Olivier Bolduc2, Caroline Proulx2, William D. Lubell2, James Fethiere3, Jean-François Masson2, Huy Ong1. 1Fac. of Pharmacy, 2Dept. of Chemistry, et 3Institut de Recherche en Immunologie et en Cancérologie, University of Montréal, Québec, Canada

Purpose: CD36, a class B scavenger receptor, is expressed at the surface of many cell types including macrophages, endothelial cells of the microvasculature and retinal pigment epithelium

(RPE). This multiligand receptor is implicated in many important physiological functions, including internalisation by macrophages of modified lipoproteins, phagocytosis of outer segment of photoreceptors or by mediating the antiangiogenic effect with its natural ligand, thrombospondin I. Our previous work on EP80317, a GHRP prototype, has shown that synthetic ligands could induce the reduction of atherosclerotic lesions mediated by CD36. In order to develop a new class of selective ligands to CD36 for structure-activity study, a sensitive new screening approach is required. We propose in this context the use of surface plasmon resonance for the screening of these ligands.
Methods: Human CD36 ectodomain (hCD36ED) was cloned into a donor vector to produce the bacmid, precursor of the recombinant virus to infect High Five insect cells. The protein expressed was collected from media and purified on Nickel column (Poros, Applied Biosystems) and then on anti-Flag affinity gel (Sigma-Aldrich). The highly purified receptor was immobilised on a surface plasmon resonance surface, by a chelated copper ion at the end of a peptidomimmetic fixed on a gold surface by a thiol group. Peptidic ligand solutions, diluted in PBS at concentrations varying from 30 µM to 100 nM, were passed through the surface.
Results: The expression of human CD36 ectodomain gave a 65 kDa protein which was purified to homogeneity (>90%) by two different affinity steps. The use of the SPR methodology allows the determination of the affinity constants of the azapeptides tested: EP80317 (8,2 x 10^{-8}M), DBG178 (2,2 x 10^{-8}M), CP-3(iv) (3,8 x 10^{-7}M), CP-2B(i) (1,4 x10^{-6}M).
Conclusion: We show here SPR as a new methodological approach by SPR to characterize the CD36 synthetic ligands. SPR could be also applied for high throughput screening of synthetic ligands of this scavenger receptor.

100. EP 80317, A Selective Ligand of CD36 Fatty Acid Translocase, Reduces Plasma Non-esterified Fatty Acid Following Reperfusion of Ischemic Heart

David N. Huynh1*, Valérie L. Bessi1*, Sébastien M. Labbé2, André C. Carpentier2, Huy Ong1, Sylvie Marleau1. 1Faculté de pharmacie, Université de Montréal, QC; 2Département de médecine, Faculté de médecine, Université de Sherbrooke, QC.

Purpose: Experimental evidence points to a central role of CD36 in facilitating myocardial fatty acid (FA) uptake and metabolism in the heart. Reperfusion of ischemic heart is associated with a burst in FA oxidation at the expense of glucose oxidation, which is associated with myocardial lactate accumulation, acidification and dysfunction. Our recent studies showed that a pretreatment of mice with a selective CD36 ligand, EP 80317, protected mice from myocardial ischemia-reperfusion (I/R)-induced tissue injury. Furthermore, reduced infarct areas were associated with both reduced plasma non-esterified FA (NEFA) and myocardial NEFA global uptake. The present study aimed to determine the cardioprotective mechanisms of EP 80317 in reducing circulating NEFA.
Methods: C57BL/6 mice were treated daily with either EP 80317 (289 nmol/kg) or 0.9% NaCl subcutaneously for 2 weeks prior to subjecting the mice to 30 minutes of ischemia by ligation of the left coronary artery. Hearts were then reperfused for either 6 or 48 hours to measure the expression levels of adipogenic and metabolic genes at the mRNA and protein levels in epididymal fat pads, liver, and skeletal muscles.
Results: EP 80317 treatment increased mRNA levels of the adipogenic and lipid storage genes, including C/EPBα, PEPCK, and perilipin, whereas no change in CD36 nor UCP1 were observed at 6 hours following reperfusion. In contrast, no change in adipose tissue gene expression was observed at 48 hours of reperfusion. In addition, no change in CD36, PPARα, PGC-1α, CPT1, nor SREBP-1c gene expression levels were detected in the liver or skeletal muscle in EP 80317-treated mice. Altogether, these results suggest a transient attenuation in adipose tissue lipolysis, with the upregulated genes mainly contributing to triacylglycerol storage. Moreover, no change in the activation of HSL was observed in EP 80317-treated mice.
Conclusion: The cardioprotective effects observed following EP 80317 treatment may be related, at least in part, to its effect on peripheral adipose tissue, through promoting a transient storage of NEFA as triglycerides, thereby leading to a transient decrease in plasma NEFA and myocardial NEFA uptake.
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*Co-first authors

101. Effects of TNF-a Gene Knockout on Basal and Antipsychotic-induced *Nur77* Expression in the Mouse Brain

Soriya Kvann[1], Gil M. Lewitus[2], David Stellwagen[2] and Daniel Levesque[1]. [1]Faculty of Pharmacy, University of Montreal, Montreal, QC, Canada; [2]Centre for Research in Neuroscience, McGill University, Montreal, QC, Canada

Purpose: Tumor necrosis factor (TNF-α) is a cytokine involved in inflammatory and neuronal signaling. Recent studies have demonstrated and important reciprocal relationship between TNF-α and *Nur77*, a transcription factor of the orphan nuclear receptor family, in the periphery. In the central nervous system (CNS), *Nur77* (NGFI-B, NR4A1) is closely related to dopamine neurotransmission. But, the relationship between TNF-α and *Nur77* in the CNS has never been explored. To investigate the putative relationship between TNF-α and *Nur77* in the central dopamine system, we evaluated *Nur77* mRNA levels in vehicle- and haloperidol-treated (a dopamine D_2 antagonist, conventional antipsychotic) wild type and TNF-α knockout mice.
Methods: An acute injection of vehicle (0.5 ml/kg) or haloperidol (0.5 mg/kg) has been performed in groups of wild type and TNF-α knockout mice (N=5 per group). Animals were sacrificed 1 hour after the injection and the brains were rapidly frozen. Brain sections (12 um) were collected in a cryostat apparatus (-20°C), put on glass slides and maintained at -80 °C until used. Brain areas analyzed included midbrain regions substantia nigra (SN) and ventral tegmental area (VTA) (containing dopamine synthesizing neurons), and forebrain areas receiving these dopaminergic inputs, namely, the prefrontal and cingulate cortices, nucleus accumbens shell and core, and the striatum (divided in 4 subterritories, dorsomedial, dorsolateral, ventromedial and ventrolateral areas). *Nur77* mRNA levels were quantified by *in situ* hybridization using a selective *Nur77* radiolabeled complementary RNA probe.
Results: A significant reduction of basal levels of *Nur77* was observed in TNF-α KO mice in the cortex, lateral striatum and nucleus accumbens core. However, haloperidol-induced *Nur77* mRNA levels were similar in wild type and TNF-α knockout mice, except in the ventrolateral striatum and SN/VTA areas, where haloperidol-induced *Nur77* expression was lower in TNF-α knockout compared to wild type mice.
Conclusion: These data suggest that *Nur77* is a downstream target of TNF-α signaling in dopamine-related areas of the CNS.
Acknowledgement: Soriya Kvann is an awardee of the National Summer Student Research Program Awards to Present Research Findings sponsored by GlaxoSmithKline Inc.

102. Effects of Vitamin D Analogs on Cyp7a1, the Rate-Limiting Enzyme in Cholesterol Metabolism

Yatong Li, Edwin C.Y. Chow, K. Sandy Pang. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Canada

Purpose: 1α,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the natural ligand of the vitamin D receptor (VDR), was found to induce murine hepatic cytochrome P450 7a1 (Cyp7a1), the rate-limiting enzyme in the metabolism of cholesterol to bile acids. It was also shown to reduce plasma and liver cholesterol levels in mice that were given a high fat/high cholesterol diet (Chow et al., unpublished data). Dietary vitamin D and $1\alpha(OH)D_3$, the (respective) precursor and intermediate prodrug of the active $1,25(OH)_2D_3$, were given to examine changes in the mRNA and protein levels of transporters and enzymes in mice.
Methods: Male, C57BL6 mice were injected with dietary vitamin D (0, 50, 250, and 500 μg/kg, ip daily x8) or $1\alpha(OH)D_3$ (0, 0.25, 1.5 μg/kg, every other day for 8 days). $1\alpha(OH)D_3$-treated mice were maintained on a high fat (42%), high cholesterol (0.02%) diet for 2 weeks. Portal and systemic blood samples, liver and kidney tissues, and ileal enterocytes were harvested. Plasma total bile acids, plasma cholesterol, and liver ALT were assayed. Real time polymerase chain reaction and Western blotting were used to determine RNA and protein expression, respectively.
Results: Dietary vitamin D produced spurious results, including the downregulation of hepatic Cyp7a1 mRNA ($P<0.05$ at 250 μg/kg) and protein ($P<0.05$). For mice on a high fat/high cholesterol diet, the higher 1.5 μg/kg dose of $1\alpha(OH)D_3$ significantly ($P<0.05$) decreased dietarily-elevated plasma cholesterol levels and increased bile acid concentrations in the portal vein. Total mRNA expression was induced for hepatic Cyp7a1 and

other VDR target genes in the intestine (Cyp24 and Asbt) and kidney (Cyp24, Mdr1, and Mrp4). In contrast, hepatic Bsep, Mrp2, and Cyp3a11, renal FXR and Asbt, and ileal SHP were decreased. A significant correlation between Cyp7a1 mRNA and SHP mRNA was also found. While renal P-gp and Mrp4 protein levels increased with the $1\alpha(OH)D_3$ treatment, the hepatic Cyp7a1 protein levels were not significantly altered.

Conclusion: It was concluded that the dietary vitamin D precursor failed to increase Cyp7a1 mRNA and protein, likely due to its low conversion rate to $1,25(OH)_2D_3$. However, the higher dose of the intermediate precursor, $1\alpha(OH)D_3$, was able to upregulate murine Cyp7a1 mRNA but not protein expression, as the latter result may be attributed to liver toxicity and hypercalcemia.

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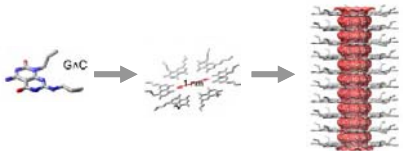
103. Influence of siRNA on the Self Assembly of Twin G□C Supramolecular Structure

Aws Alshamsan[1,2], Mounir El Bakkari[2,3], and Hicham Fenniri[2,3]. [1]Department of Pharmaceutics, College of Pharmacy, King Saud University; [2]National Institute for Nanotechnology (NINT-NRC), University of Alberta; [3]Department of Chemistry, Faculty of Science, University of Alberta.

Purpose: The self-assembly of synthetic self-complementary twin guanine-cytosine (G□C) motifs is an entropy-driven process. During the assembly, 6 G□C motifs organize in a rosette shape held by 18 H-bonds. Then, the formed rosettes further arrange in nanotubes (RNTs) by □□□ stacking. However, when used as vehicles for drug deliver, the supramolecular structure of the G□C motifs may change according to the payload to achieve thermodynamic stability. In this report, we examine the effect of small-interfering RNA (siRNA) on the supramolecular structure of G□C motifs modified with 4, 8, and 12 lysine residues i.e. K4.T, K8.T, and K12.T, respectively. **Methods:** In sterile tubes, increasing mole ratios (0.5-20) of K4.T, K8.T, and K12.T were incubated with 1 mole of siRNA for 45 minutes in 37°C in unbuffered water. Thereafter, siRNA binding was determined by agarose gel electrophoresis and the binding capacity was calculated based on the optical density of the siRNA band. Scanning electron microscopy (SEM) was then conducted to study the morphology of the siRNA:Kn.T supramolecular structures and was compared to siRNA-free Kn.T controls.

Results: Gel electrophoresis studies showed complete binding of siRNA with 20 moles of K4.T and 10 moles of K8.T and K12.T. Moreover, in K8.T and K12.T groups, siRNA bands were partitioning at two molecular weight levels in the gel indicating the possibility for new-entity formation. This observation was recorded for K8.T and K12.T starting from mole ratios of 2.5 and 1, respectively. SEM images showed that free K4.T forms RNTs that are around 5.02 ± 0.5 nm in diameter and about 500 □m in length. Free K8.T also formed RNTs but that of shorter length (300 □m) and smaller diameter (6.05 ± 0.1 nm) as compared to K4-RNTs. On the other hand, free K12.T was not able to self-assemble into supramolecular structure. Upon siRNA incubation, K4-RNTs formed bundles that further aggregated into networks. Similarly, K8-RNTs aggregated into bundles with appearance of spherical structures within the aggregates. K12.T started to show RNT-bundle formation upon siRNA incubation. However, nanospheres of siRNA:K12.T were independently detected. The size of the nanospheres was inversely proportional to the ratio of K12.T to siRNA used.

Conclusion: The presence of siRNA can serve as an initiator for Kn.T self-assembly. Moreover, the size and dimension of Kn.T supramolecular structure can be controlled based on the ratio of siRNA used, which is an advantage as a passive drug targeting strategy.



104. Angiotensin II Regulation of Calcium Current in Mouse Ventricles

Sophie Mathieu[1, 2], Katy Rivard[1, 2], Nabil El Khoury[1, 2], Pierre Paradis[3], Mona Nemer[4], Céline Fiset[1, 2]. [1]Research Centre, Montreal Heart Institute, Montréal, QC; [2]Université de Montréal, Faculty of Pharmacy, Montréal, QC; [3]Lady Davis Institute, Montréal, QC; [4]Ottawa University, Ottawa, ON,

Canada.

Purpose: High levels of angiotensin II (ANGII) can lead to cardiac hypertrophy, heart failure and arrhythmias. These effects are thought to be mediated by type 1 ANGII receptor (AT1R). Cardiac specific overexpression of AT1R in mice is associated with the development of cardiac remodelling and arrhythmias. Many studies have demonstrated that AT1R signalling modulates L-type Ca^{2+} channels ($Ca_v1.2$) and that T-type calcium channels ($Ca_v3.1$ and/or $Ca_v3.2$) are re-expressed in hypertrophy. Since alterations in Ca^{2+} currents may be involved in the occurrence of cardiac arrhythmias, we decided to examine the effect of AT1R overexpression on L-type (I_{CaL}) and T-type (I_{CaT}) Ca^{2+} currents.
Methods and Results: Ventricular myocytes were isolated from male (M) and female (F) of 50-day (50d, without hypertrophy) and 6-month (6m, with hypertrophy) old AT1R mice. Whole-cell voltage-clamp recordings obtained at 37^0C revealed that at 50 days, I_{CaL} density is similarly decreased between male and female AT1R mice by 51% and of 52% respectively compared to their littermates controls (CTL) (at 0 mV (pA/pF), 50d: M: CTL: -10.7±2.1, n=5; AT1R: -5.3±0.6, n=7, $p<0.05$; F: CTL: -13.4±0.7, n=6; AT1R: -6.4±2.9, n=4, $p<0.05$). At 6 months, I_{CaL} was less decreased in male than female AT1R mice 30% and 62%, respectively (6m: M: CTL: -10.3±0.6, n=5; AT1R: -7.2±0.3, n=8, $p<0.05$; F: CTL: -10.6±0.5, n=21; AT1R: -4.0±0.3, n=3, $p<0.05$), suggesting that the reduction of I_{CaL} in female AT1R mice may be aggravated by hypertrophy. Moreover, consistent with the electrophysiological data, there was a ~30% reduction in $Ca_v1.2$ mRNA in all groups of AT1R mice compared to their respective controls. The very low density of the ventricular I_{CaT} was similar between the different groups (CTL and AT1R of both ages and both sexes) (i.e., 1.0±0.2 pA/pF at -45 mV).
Conclusion: All together, these findings suggest that the reduction of I_{CaL} and $Ca_v1.2$ expression produced by ANGII exposure is mediated by the stimulation of the AT1 receptor and is independent of cardiac remodelling.

105. Effects of Pro-inflammation Cytokines on L-type Calcium Currents in Mouse Cardiomyocytes

Nabil El Khoury[1,2], Céline Fiset[1,2]. [1]Research Center, Montreal Heart Institute, Montreal, Quebec; and [2]Faculty of Pharmacy, Université de Montréal, Montréal, Quebec, Canada

Purpose: Pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and (IL-6) have been implicated in the pathogenesis of heart failure. A high level of cytokines has also been reported in patients suffering from arrhythmias. Additional studies showed that pro-inflammatory cytokines can affect cardiac contractile function. Together these observations support the notion that elevated levels of pro-inflammatory cytokines contribute to cardiac rhythm disturbances associated with cardiac pathologies.
Since L-type calcium current (I_{CaL}) also plays a critical role in electrical and mechanical functions of the heart, the purpose of this study was to determine the effect of chronic exposure to pro-inflammatory cytokines (TNF-α, IL-1β or IL-6) on I_{CaL} density and its underlying α-subunit, Cav1.2.
Methods: Neonatal ventricular myocytes were isolated from 1-2 day old CD1 mice and maintained in primary cell culture for 24 hours under control conditions or in the presence of 30 pg/ml of TNF-α, IL-1β or IL-6. We used patch-clamp technique in the voltage-clamp mode to record I_{CaL} and quantitative real-time PCR (qPCR) to measure mRNA expression of $Ca_V1.2$ in cultured control- and cytokine-treated myocytes.
Results: The patch-clamp experiments revealed that the density of I_{CaL} (expressed in pA/pF) was decreased by 36% in IL-1β-treated ventricular myocytes compared to controls (at 0 mV, CTL**:** -6.2±0.5, n=17 and, IL-1β: -4.0±0.5, n=19 $p<0.05$). In contrast, the density of I_{caL} was comparable between control, TNF-α and IL-6 groups. Ventricular myocytes were also exposed to the combination of IL-1β and TNF-α. In the presence of both cytokines, the reduction of I_{CaL} (at 0 mV, CTL**:** -5.9±0.5, n=18; IL-1β+TNF-α: -3.9±0.4, n=24, $p<0.05$) was comparable to that of IL-1β alone, ruling out any potential synergistic effect of between IL-1β and TNF-α on I_{CaL}. qPCR results showed no significant decrease in $Ca_V1.2$ expression in cytokine-treatment myocytes compared to the untreated controls.
Conclusion: Overall, this study shows that although

TNF-α and IL-6 do not affect Ca^{2+} current in mouse ventricular myocytes, pathologically relevant concentration of IL-1β significantly decreases the density of I_{CaL} without affecting the expression of Cav1.2.

106. Screening of Bark Extracts from Canadian Wood Species and Investigation of their Skin Anti-aging Activities for Dermocosmetic Applications

Mariana Royer, Maria Prado, Martha-Estrella García-Perèz, Papa Niohkor Diouf, Tatjana Stevanovic. [1]Centre de Recherche sur le Bois, Faculté de foresterie et de géomatique, Université Laval, QC; [2]Serex, Amqui, Canada.

Purpose: Forest biomass valorization strategies are of particular interest for the development of the Canadian forest industry. Bioactive polyphenols, present in large quantities in the barks, can be extracted with "green" solvents and used for dermocosmetic applications. The present study aims to investigate the skin anti-aging activities of twelve extracts obtained from the bark of six Canadian wood species: Picea mariana (BS), Pinus banksania (JP), Abies balsamea (BF), Betula alleghaniensis (YB), Populus tremuloides (TA) and Acer rubrum (RM). These extracts were tested for lipidic peroxidation, tyrosinase and elastase inhibition and antimicrobial activity. The toxicity of the most active extract on normal human keratinocytes (NHK) was also determined.
Methods: Bark extracts were obtained by hot water extraction and maceration using90 % aqueous ethanol. The elastase and tyrosinase inhibitory activity of these extracts as wells as their ability to inhibit lipidic peroxidation (0.05-1.0 mg/ml) were analyzed by spectrophotometric methods and compared to pure compounds (acorbic acid, trolox, epigallocatechin, kojic acid) and the standardized French maritime pine bark extract (Oligopin®) The antimicrobial activities of the extracts was done against two non- pathogenic bacteria: Escherichia coli (Gram-) and Listeria ivanovii (Gram +). Minimal toxic dose (IC_{90}) of the most active extract on NHK was determined by MTT assay after 24 h of exposure.
Results: Results show that ethanolic extracts were more active against lipidic peroxidation than their aqueous analogs. Among aqueous extracts, BS was the most active extract with and IC_{50}= 158.7 +/- 52 µg/mL lower than ascorbic acid but higher than Oligopin®, standard extract. Among ethanolic extracts, RM was the most active with an IC_{50}= 86.8 +/- 6.3 µg/mL lower than all positive references. Anti-enzymatic assays show that RM extracts were the most active among all extracts, the ethanolic one showing the highest activities. This extract also exhibited high antibacterial activity, in particular against L.ivaonvii, Gram+. Minimal toxic doses of RM ethanolic extract on NHK after 24 h was determined to be 398.02 ± 151.12 µg/mL.
Conclusion: Canadian bark extracts could be considered for applications in dermocosmetics acting as antioxidant and/or skin anti-aging agents. RM bark extracts show the highest activities at concentrations lower than the minimal toxic dose.

107. Development of an Oral Ecosystem Model to Study the Effects of the Microflora on Oral Health

Shyamali Saha [1,2], Catherine Tomaro-Duchesneau[1], Meenakshi Malhotra[1], Hani Al-Salami[1], Imen Kahouli[1], Maryam Trabizian[2], Satya Prakash[1]*. [1]Biomedical Technology and Cell Therapy Research Laboratory, Department of Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, Quebec, Canada; [2]Faculty of Dentistry, McGill University, Montreal, Quebec, Canada

Purpose: Oral health is determined by the oral cavity's thousands of resident microorganisms. Three main oral disorders are dental caries (DC), gingivitis and periodontitis, with the oral microbiota playing an important role in the initiation and progression of these. One important causal agent of DC is *Streptococcus mutans*. Gingivitis is characterised by inflammation of the gingiva which can progress further to involve the supporting structures of the tooth, such as the alveolar bone. In this case, the disorder is then termed periodontitis. A number of factors are involved in disease progression, with oral hygiene, oral ecosystem variation, age and smoking all playing a role in disease initiation and progression. *In vitro* studies involving the effect on resident microorganisms on the initiation and progression of oral disorders remain limited. The goal of this research is to develop an *in vitro* model of the oral ecosystem, using key oral resident bacteria as characterising tools, to allow for a more cost- and time-effective method to develop therapeutics.

Methods: The oral ecosystem was developed using a number of simulated saliva formulations (carbohydrate sources, amino acids, salivary enzymes, vitamins, etc.). The concentrations of these were optimized using *S. mutans* and LGG as indicator organisms present in the oral microflora. Factors such as pH and salivary flow rates were also modulated for system optimization. Viability of bacterial strains was determined by colony counting methods and RT-PCR.
Results: The *in vitro* model was developed and tested under different sugar concentrations and pH, to represent the environmental changes of the oral cavity. Under the simulated conditions, *S. mutans*, the indicator organism, was shown to remain stable over 72 hours in this system, demonstrating that the conditions are indeed optimized for this strain. Another resident microbial strain, a Lactobacillus (LGG) was shown to also remain viable in the oral ecosystem model. Fluctuations of sucrose concentrations, present following food intake, were shown to greatly affect the growth of *S. mutans*.
Conclusion: An *in vitro* model was developed to simulate the oral ecosystem. *S. mutans* to represent the cariogenic oral microflora was characterised in this system with the bacteria remaining stable as a biofilm for 72 hours. This model can further be used to investigate the capacity of therapeutics to prevent disorders such as caries formation.
Acknowledgement: The authors would like to acknowledge a Canadian Institute of Health Research (CIHR) grant (MOP 264308) to Dr. S. Prakash.

108. Inflammation Alters the Balance Between ACE and ACE-2 Protein Expression in Rat Heart

Mahtab Tavasoli, Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

Purpose: It is apparent that both the inflammatory condition and nonsteroidal anti-inflammatory drugs (NSAIDs) including the selective COX-2 inhibitors adversely influence the cardiac function. New findings in our lab suggest that inflammation alters the expression of genes that encode cyclooxigenase-1 (COX-1), COX-2, angiotensin converting enzyme (ACE) and ACE-2 in rat heart. The variations in tissue distribution of these enzymes are among the most widely accepted explanations for the cardiovascular risk associated with these diseases and drugs. In order to discover the influence of COX and ACE inhibition on cardiovascular function, As the first step, we studied the effect of inflammation on the expression of ACE, ACE-2, COX-1 and COX-2 proteins in 4 groups (n=4/group) of inflamed pre-adjuvant (AA) and Control (healthy) male Sprague-Dawley rats.
Methods: The inflamed group received 0.2 ml of 50 mg/ml Mycobacterium butyricum suspended in squalene into the tail base. On day 12, rats were euthanized and their hearts were excised. ACE, ACE-2, COX-1 and COX-2 protein expression in rat heart was determined by Western blot.
Results: The Western blot experiment revealed a strong trend towards ACE-2 reduction ($p=0.056$) and no change in ACE in AA rats as compared to Control. There was a significant increase in the gap between ACE and ACE-2 expression in the rat heart. Similar to what has been previously reported about COX-1 mRNA, COX-1 protein expression had a trend towards down regulation in the heart of Pre-AA rats that did not reach significance. Inflammation resulted in a significant increase of COX-2 protein in the rat heart.
Conclusion: ACE-2 has been found to provide negative feedback of RAS and protection of the heart and kidneys. Disruption of the balance between ACE and ACE-2 observed in inflammation maybe at least in part, involved in the cardiovascular complications seen in patients with inflammatory diseases. Pathophysiological significance of the observed changes of COX enzymes in AA rats needs to be studied more; however the altered balance may play a role in the pathogenesis of the cardiovascular complications observed in inflammatory diseases.

109. Signalling Pathways of Orexigenic Peptides QRFPs in the Regulation of Peripheral Lipolysis

Mukandila Mulumba[1], Christian Jossart[1], Sylvie Marleau[1] and Huy Ong[1,2]. 1 Faculty of Pharmacy and 2 Department of Pharmacology, University of Montreal, Quebec, Canada

Background: The orexigenic RFamide peptides QRFP-43 and -26 have been reported to bind to GPR103 receptors in hypothalamic regions involved in feeding regulation. We have shown that fat tissues as well as 3T3-L1 adipocytes express both QRFPs and its receptors. QRFPs were found to inhibit

isoproterenol (ISO) induced-lipolysis in nanomolar ranges. In the regulation of lipolysis process, beta-adrenergic receptor activation by catecholamines promotes adenylate cyclase activity leading to the production of intracellular cAMP and to the activation of PKA. Mechanisms of controlling PKA activity involve phosphodiesterases (PDEs) which reduce cAMP levels and thus PKA activity. It has also been shown that PKC activation leads to the induction of lipolysis in adipocytes.
Objective: To determine the signalling pathways involved in the anti-lipolytic effects of QRFPs using differentiated 3T3-L1 adipocytes.
Methods: Differentiated 3T3-L1 cells were pre-incubated with QRFP-43 (10 nM) for 30 min. PKA activation promoting lipolysis was induced by treating 3T3-L1 adipocytes either with ISO (300 nM, 30 min) or forskolin (2.5 μM, 2h) or 8-bromo (Br)-cAMP and dibuyryl (DBT)-cAMP (250 μM, 2h) at 37°C. PKC promoting lipolysis was elicited with phorbol 12-myristate 13-acetate (PMA, 4 μM) for 4h. After the incubation period, culture medium was collected for free glycerol measurement and lipolysis index (% of lipolysis induced by ISO) was determined.
Results: QRFP-43 elicited a 21% ($P<0.01$) and 23% ($P<0.05$) inhibition of ISO and forskolin induced-lipolysis, respectively. In order to define the role of PDEs, lipolysis was induced by 8-Br-cAMP and DBT-cAMP (a non-hydrolysable cAMP analog). QRFP-43 inhibited both 8-Br- and DBT-cAMP induced lipolysis by 24 and 43% ($P<0.01$), respectively. In contrast, insulin used as positive control inhibited 8-Br-cAMP induced-lipolysis by 45% ($P<0.01$) but failed to inhibit DBT-cAMPinduced lipolysis. PMA induced lipolysis was inhibited by 27% ($P<0.05$) following QRFP-43 treatment. In presence of PKC inhibitor GF109230x (2 μM), QRFP-43 treatment completely inhibited lipolysis stimulated by PMA. GF109230x treatment alone inhibited by 69% ($P<0.001$) PMA induced lipolysis.
Conclusion: These results suggested that QRFP-43 inhibited both lipolysis induced through PKA and PKC activation. In addition, QRFP-43 elicited anti-lipolytic effect on ISO stimulation may be independent of PDEs activation.
Supported by an educational grant of Aeterna Zentaris Inc.

Clinical Sciences & Pharmacy Practice

110. Inflammasome Modulated by Monotherapy in Chronic Hepatitis C Patients

M.G. Neuman, G.G. Katz, A. Patel, A.A. Baig, G. Moussa, T. Mazzulli, I.M. Malkiewicz. Departments of Pharmacology & Toxicology, and Laboratory Medicine, Faculty of Medicine, University of Toronto, and In Vitro Drug Safety and Biotechnology, MaRS, and Department of Microbiology, Toronto, Ontario, Canada

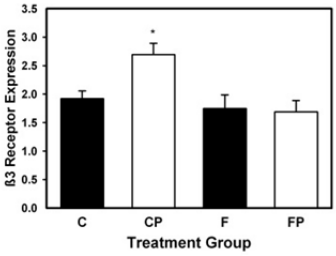
Pegylated-interferon (PEG-IFN-a) monotherapy is needed in hepatitis C virus (HCV) infected-patients not-tolerating ribavirin.
Objective: to evaluate the inflammasome in HCV, to correlate serum apoptosome, pathogen-associated-molecular-pattern (PAMP), plasminogen-activator-inhibitor 1(PAI-1), tumour necrosis factor-alpha (TNFa□, and transforming growth factor-beta (TGFβ) levels with the severity of HCV, and the responses to PEG-IFNa-2b.
Methods: 180-non-cirrhotic patients received 0.5, 1.0 and 1.5 mg/kg/week PEG-IFN for 48 weeks. Each group was stratified: sustained-response-SR [HCV-RNA undetectable 6 months after the end-of-therapy (ET)], relapse-response-RR (HCV-RNA undetectable ET) or no-response-NR (detectable HCV-RNA at ET). The HCV-RNA was monitored by TaqMan-Amplicor PCR (Roche Diagnostic). Inflammasome-levels were measured by ELISA. Student-t-test with Bonferonni correction determined the significance between the groups.
Results: Of 180 patients; 3 had 0 histological-activity-index (HAI), 47-mild, 121-moderate and 9-high; and had Metavir-fibrosis (MF0-5; MF1-152; MF2-13; MF3-10). A good correlation was seen between the HAI and TNF-a levels ($r=0.92$, $p<0.001$) in all the patients ($r=0.85$; $p<0.001$). TGFβ increased significantly with the severity of fibrosis. TNFa and apoptosis were lower at the base-line in SR versus RR and NR. There is a correlation between the HCV-RNA and HAI-reduction, as well as the decrease of TNF□ and apoptosis. Regardless the dose of therapy, TNF-a and TGFβ decreased significantly in SR-patients versus their initial values. PAMP and PAI-1 did not demonstrate differences between the doses.
Conclusion: Severity of liver disease is lowered in

patients after therapeutic intervention regardless of their response to therapy. Low baseline serum TNFa and apoptosome are predictors for SR. PEG-IFN-a reduces inflammasome contributing to reduce fibrosis.

111. Medication Incidents Involving Psychotropic Drugs: An Aggregate Analysis

Calvin Poon, Roger Cheng, Sanaz Riahi and Sylvia Hyland. Institute for Safe Medication Practices Canada, Toronto, Ontario

Purpose: Mental health disorders pose a significant burden to the Canadian health care system, both from an epidemiological and financial perspective. Given the prevalence and financial burden of treating mental disorders, it is imperative that there is a strong emphasis on safe medication practices when managing psychotropic medications. By analyzing the incidents involving psychotropic medications, systems based contributing factors could be identified which can inform quality improvement initiatives.
Method: Medication incidents from the ISMP Canada database were collected from October 7, 2000 to July 29, 2009. Incidents with an outcome of harm or death involving antipsychotics, antidepressants, antimanic agents, sedatives and hypnotics were included. A quantitative analysis was conducted to provide an overview of various trends such as the severity of outcome. A qualitative analysis was conducted to identify recurrent themes and contributing factors.
Results: A total of 88 incidents were included in the analysis; 82 had an outcome of harm, and 6 had an outcome of death. These incidents occur at different patient care settings including the hospital, community pharmacy and long term care. A number of themes have been identified and were classified under the patient care setting where the incident occurred. For example, in the hospital setting, the themes identified included “multiple medications”, “incorrect dose”, “incorrect patient”, “incorrect medication”, “change in order”, “transitions of care” and “dose omission”. Incidents classified under these themes were further analyzed to identify potential contributing factors. Examples of contributing factors identified included complicated instructions in orders, pre-pouring of medications and the lack of a systematic medication reconciliation process.
Conclusion: The results of this analysis can provide insights into areas for system improvements for the use of psychotropic medications. The potential contributing factors identified provide a solid foundation for the development of solutions to minimize the recurrence of similar incidents.



Acknowledgement: Calvin Poon is an awardee of the National Summer Student Research Program Awards to Present Research Findings sponsored by GlaxoSmithKline Inc.

112. Danger Signals in Nevirapine-Induced Skin Rash

Xiaochu Zhang and Jack Uetrecht. Faculty of Pharmacy, University of Toronto, Toronto, ON

Purpose: Nevirapine (NVP) causes serious skin rashes and hepatotoxicity. It also causes an immune-mediated skin rash in rats with characteristics very similar to the skin rash in humans but not hepatotoxicity. There is strong evidence that this is due to 12-OH-NVP, which is further metabolized to a sulfate and binds to proteins in the skin. In contrast most of the covalent binding in the liver appears to involve oxidation of the same methyl group leading directly to a reactive quinone methide. In this study we examined the effects of NVP and 12-OH-NVP on gene expression in both the liver and skin.
Methods: Female BN rats were with NVP or 12-OH NVP, changes in gene expression in both the liver and skin were determined with Affymetrix gene chips. Elisa was used for serum study of a significant gene.
Results: In the liver, more genes were significantly changed at 6 hrs than at 12 hrs. ER-stress and immune-response related genes were significantly up-regulated by NVP and DNVP, while 12-OH-NVP induced other genes, including CD36, a scavenger receptor on macrophages. Although both NVP and 12-OH-NVP induced changes in the liver, the list of genes was very different. This is not surprising because only NVP can be directly

oxidized to the reactive quinone methide. NVP induced genes such as ZAP70, which is associated with control of immune tolerance, and these animals do not develop hepatotoxicity. In contrast, much more significant genes were up-regulated in the skin with 12-OH-NVP treatment in comparison with NVP, which is consistent with the hypothesis that the rash is induced by 12-OH-NVP. Some top genes up-regulated by 12-OH-NVP were Trim-63, which is induced by interferons, S100a7a, which represents a danger signal, and IL22-RA2, which is associated with autoimmunity. Elisa of IL-22RA2 showed its elevation in rat serum after NVP treatment in food for 5 days, which is close to ear redness. Methods and Results: 6 and 12 hr after treatment.
Conclusions: These data support the hypothesis that the 12-hydroxylation pathway is involved in NVP-induced skin rash and provides clues to the effects of these drugs in the liver and skin.

Pharmacokinetics & Pharmacodynamics

113. Steady-State Bioequivalence Potential of a New Extended-Release Formulation Determined by Clinical Trial Simulations

Philippe Colucci[1], Corinne Seng Yue [1,2], Murray P. Ducharme[1,2], [1]Cetero Research, Cary, NC, [2]Université de Montréal; Montréal, QC.

Purpose: To determine the fasting steady-state equivalence potential of a new super generic formulation of an antihistamine given twice a day versus the six times a day reference formulation in adults and young adults based on single dose data.
Methods: In a single-dose crossover study performed in 58 adults and young adults (age 12 to 17 years), the PK of a test q12h extended-release suspension given once (three times the reference strength) was compared to a q4h reference liquid given three times (Time 0, 4 and 8 hours). Fasting PK parameters were calculated using noncompartmental approaches. Additionally, compartmental modeling and simulations were performed with ADAPT 5® to determine the two formulations' pharmacokinetics and to assess the bioequivalence potential with simulated steady-state studies (a total of 10). Noncompartmental parameters were calculated for the simulated studies. Steady-state bioequivalence was determined using FDA guidelines based on parameters AUC0-t(ss), $Cmax_{(SS)}$ and $Cmin_{(SS)}$.
Results: The drug's pharmacokinetics was best described by a 2-compartment model with 6 absorption peaks for the test formulation and 2 absorption peaks after each reference dose. Results for adults and young adults were similar. Based on steady-state simulations, it is expected that the 90% confidence interval for the PK parameters AUC0-t(ss) and $Cmax_{(SS)}$ would meet the 80-125% bioequivalence criteria, indicating that the rate and extent of exposure at steady-state would be equivalent. However, these simulations predicted that the ratio for $Cmin_{(SS)}$ would be 71% (below the usually accepted 80%). These results show that there should be no safety concerns at steady-state as exposure for the test product administered q12h is equivalent to the reference product administered q12h. Although simulated $Cmin_{(SS)}$ would be slightly lower for the test regimen, they should remained above the minimum efficacious concentrations throughout the dosing interval.
Conclusions: This study demonstrates the utility of clinical trial simulations to help decision making in the drug development process.

114. Phentolamine Treatment Prevents Increases in Blood Pressure in Fructose fed Rats

Kangbin Zhou, Violet G. Yuen, Ujendra Kumar and John H. McNeill. Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia

Purpose: Hypertension has been characterized as a major risk factor involved in the metabolic syndrome and poses a challenge in the development of effective drug therapy. The cause of this elevated blood pressure is unclear. A variety of anti-hypertensive drugs have been shown to prevent the increase in blood pressure in rats fed fructose. Such animals also show other signs of the metabolic syndrome including increased insulin levels and decreased insulin sensitivity. In this study, phentolamine, a non-selective α adrenergic receptor antagonist, was used to examine the prevention of a fructose-induced elevation in blood pressure and the possible mechanism(s) involved.
Methods: Twenty four Wistar rats were assigned into four experimental groups: control (C), control

phentolamine treated (CP), fructose (F), and fructose phentolamine treated (FP). Fructose diet (60%) and phentolamine treatment (5 mg/kg) were started at six weeks of age. All animals were terminated when the systolic blood pressure (SBP) in F group showed a significant increase. The hearts from 50% of the animals per group (n=3) were frozen in liquid nitrogen for Western Blot analysis. The other 50% (n=3) were fixed in 40% paraformaldehyde for immunohistochemical analysis.

Results: Phentolamine treatment prevented the increase in SBP in the FP group (F: 113±3.9 mmHg vs FP: 99±1* mmHg, $p < 0.05$) with no significant effect on SBP in the control groups. There was a significant decrease in insulin sensitivity in the fructose fed rat groups that was not effected by phentolamine treatment (C: 17.9±1.8, CP: 21.0±0.9, F: 8.1±0.8* and FP: 8.1±0.9*, $p < 0.05$). Western Blot and immunohistochemical analyses revealed an increased expression of cardiac β_3 receptors in the CP group. Currently, we are investigating the expression of β_1 and β_2 receptors in cardiac tissue as well as the level of phosphorylation of downstream targets such as protein kinase A (PKA) and Ca^{2+}/calmodulin kinase II (CaMKII).

Conclusion: Treatment with the non-specific α-adrenergic receptor antagonist, phentolamine, did prevent the increase in blood pressure seen in the metabolic syndrome with no effect on plasma glucose, plasma insulin or insulin sensitivity. The data indicate the prevention effect is due to receptor blockade. Intriguingly an increase in the expression of ß3 receptors has been seen with phentolamine. Studies on the other ß receptors are currently ongoing.

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115. Evidence of Involvement of the Intestinal Microflora in Low Oral Bioavailability of Glucosamine

Alyaa Ibrahim and Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada.

Purpose: The purpose of the study was to investigate the role of the intestinal flora on glucosamine oral bioavailability.

Methods: 14 rats were randomly assigned into two groups (n=7). The antibiotic treated group was given antibiotic mixture of 100 mg neomycin, 50 mg tetracycline and 50 mg bacetracin, orally, twice daily for two days, while, the control group was given saline by the same regimen. In the third day of the experiment, the two groups were orally administered glucosamine HCl equivalent to 200 mg/kg glucosamine. Blood samples were collected over 24 h post dose. Feces were collected at the end of the experiment, softened by addition of 25 mL water, vertex and centrifuged. The supernatant was then analyzed directly. In vitro degradation of glucosamine by feces was studied by incubation of glucosamine HCl equivalent to 1.25, 2.5, 5 and 10 mg glucosamine with 0.5 g of rat feces overnight, the samples were then analyzed for glucosamine.

Results: Treating the rats with antibiotics results in non significant doubling of the mean AUC_{0-9} from 12.7±18.8 μg.h/mL in controls to 25.6±6.7 μg.h/mL in the treated group, and the mean Cmax from 5.94 $\pm$ 2.73 μg/mL in controls to 10.76 $\pm$ 7.9 μg/mL in the treated group. On the other hand, a pronounced and significant increase in the % of oral dose excreted unchanged in the feces of the treated group (12.6 $\pm$ 4.1%) was found compared to that of the control group (0.09 $\pm$ 0.31%). The in vitro incubation in the presence of feces resulted in almost 50% disappearance of glucosamine.

Conclusion: Glucosamine is subjected to significant degradation by the intestinal flora that may at least in part, explain the low bioavailability of the compound.

116. Hepatocellular Study of Repellent DEET, Sunscreen Oxybenzone and Their Metabolites

Daryl Fediuk, Yufei Chen, Frank Burczynski, Xiaochen Gu. Faculty of Pharmacy, University of Manitoba, Winnipeg, MB, Canada.

Purpose: Concurrent application of the repellent DEET and the sunscreen oxybenzone (OBZ) enhances the disposition of the two compounds. This study investigated the viability of rat liver cells from exposure to DEET, OBZ and their subsequent metabolites.

Methods: Rat hepatoma cell line 1548 was exposed to i) DEET and OBZ, ii) two metabolites of DEET, *N,N*-diethyl-*m*-hydroxymethylbenzamide (DHMB) and *N*-ethyl-*m*-toluamide (ET), and iii) three metabolites of OBZ, 2,4-dihydroxybenzophenone (DHB), 2,2´-dihydroxy-4-methoxybenzophenone (DHMB) and 2,3,4-trihydroxy-benzophenone

(THB), at pre-determined concentrations of 0.1 µg/ml, 1 µg/ml and 10 µg/ml, applied either alone or in combination. The cells were incubated in their respective media for 24, 48 and 72 hours. After each interval of exposure time, the viability of cells was assessed by diluting the wells with WST-1 reagent and measuring the absorbance of each well at 450 nm with a microplate reader.

Results: DEET appeared to be more toxic to liver cells than OBZ. DEET at 10 µg/ml decreased cell viability by 8%, 15% and 17% after 24, 48 and 72 hours of exposure, respectively. OBZ at 10 µg/ml also reduced cell proliferation by 15% and 20% after 48 and 72 hours of exposure, respectively. Combined application of DEET and OBZ did not demonstrate more toxicity than their individual counterparts. ET was the most toxic DEET metabolite; cell viability was reduced by 9% and 34% from exposure to ET at 10 µg/ml after 48 and 72 hours, respectively. DHB appeared to be the most toxic OBZ metabolite; cell viability declined by 24% and 35% from exposure to DHB at 10 µg/ml after 48 and 72 hours, respectively. Combined exposure to all metabolites at 10 µg/ml for 72 hours led to a substantial 48% decrease in cell viabilities.

Conclusion: Hepatoma cellular studies indicated toxicity from exposure to DEET, OBZ and their metabolites, in particular at 10 µg/ml for 72 hours. Since DEET and oxybenzone mutually enhance their percutaneous permeation, the relationship between long-term exposure to these chemicals and potential hepatotoxicity should be further investigated.

117. The Effect of Traditional Cree Botanicals on the Bioavailability of Oral Anti-diabetic Therapies

Carolina Cieniak[1,2], Alexandra Fottinger[2], Rui Liu[2], Steffany Bennett[3], Brian C. Foster[2,4], John T. Arnason[1,2]. [1]Ottawa-Carleton Institute of Biology, University of Ottawa, Ottawa, ON; [2]Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, Ottawa, ON; [3]Neural Regeneration Laboratory, Faculty of Medicine, University of Ottawa, Ottawa, ON; [4]Office of Science, Therapeutic Products Directorate, Health Canada, Ottawa, ON, Canada

Purpose: To investigate the impact of traditional Cree botanicals used to treat type II diabetes on the bioavailability of common oral metaglinide and sulfonylurea class anti-diabetic therapies.

Methods: Human C2BBe1 intestinal cells, clone of Caco-2 cells, were grown on permeable membrane supports and differentiated into polarized monolayers. Absorption studies were conducted with induced and untreated cells with 1α,25 dihydroxyvitamin D_3 to induce genomic expression of drug disposition pathways. Donor solutions of 50 µg/mL of the anti-diabetic therapies repaglinide or gliclazide were prepared in combination with the ethanolic extracts of the Cree botanicals, at a concentration of 100 µg/mL, and were placed in the apical chambers of the system. Samples from the basolateral chamber were collected over a period of 3 hours and the presence of compounds detected using HPLC-DAD.

Results: Extracts from one traditional Cree botanical, AD09, showed no effect on the bioavailability of repaglinide or gliclazide in the basolateral chamber. The effect of additional Cree botanicals will be presented. HPLC peaks corresponding to known repaglinide metabolites were detected and LC/MS studies are underway to confirm their identity.

Conclusions: The *in vitro* studies suggest that that there is limited potential for traditional Cree botanicals used to treat type II diabetes to modulate the bioavailability of anti-diabetic therapies, repaglinide and gliclazide. As such, the possibility for adverse drug interactions for oral medications at the point of absorption through the intestine appears quite low.

118. Stereoselective Induction of Omeprazole Metabolism by Efavirenz

Veronique Michaud, Evan Ogburn, Nancy Thong, Abdulateef O. Aregbe, Troy C. Quigg, N Epstein, John T. Callaghan, David A. Flockhart, Zeruesenay Desta. Division of Clinical Pharmacology, School of Medicine, Indiana University, Indianapolis, IN, USA

Purpose: Omeprazole (OMP) is metabolized mainly through 5-hydroxylation (CYP2C19) and to some extent through sulfoxidation (CYP3A). OMP is administered as a racemic mixture and stereoselective disposition of OMP has been documented. We have previously demonstrated that efavirenz (EFV), an antiretroviral drug, induces the clearance of racemic OMP. The objective of our study was to investigate the stereoselective pharmacokinetics of OMP following treatment with

EFV.

Methods: 56 healthy subjects received a single oral dose of R/S-OMP (20 mg) following treatment with either one single dose (600 mg) or multiple doses of EFV (600 mg/day for 17 days). R- and S-OMP, R- and S-5OH-OMP and OMP-sulfone were measured by LC-MS/MS. Pharmacokinetic parameters were calculated using non-compartmental analysis (Kinetica, v5.0). Metabolic ratios (MR) of $AUC_{0-\infty}$ were calculated for each isomer of OMP. Data were analyzed by the paired-t test and Wilcoxon-matcheds paired test.

Results: Multiple doses of EFV caused a decrease in $AUC_{0-\infty}$ of R- (from 255±335 to 154±167 µg/L*h) and S-OMP (from 380±331 to 214±167 µg/L*h) compared to values measured after a single dose of EFV (all $p<0.001$). Thus, clearances of R- and S-OMP were increased by 41% and 35% after the EFV treatment period ($p<0.001$). $AUC_{0-\infty}$ for R- and S-5OH-OMP were decreased by 29% *vs* 31% after multiple doses of EFV ($p<0.001$). EFV multiple dosing decreased the MR of R-OMP/R-5OH-OMP, S-OMP/S-5OH-OMP, OMP/OMP-sulfone by 36% ($p=0.03$), 13% ($p=0.03$) and 40% ($p<0.0001$), respectively. Induction ratios were 1.4 for the R-5OH-OMP and 1.2 for the S-OH metabolic pathways. The extent of induction for R- and S-OMP was similar when clearances are evaluated; the estimated induction ratios for total clearances, clearances by CYP2C19 and clearances by CYP3A of R-OMP and S-OMP ranged from 1.6 to 1.8 for both OMP isomers.

Conclusions: Our results suggest that EFV induces the elimination of both isomers of OMP. The extent of induction by EFV for CYP2C19 and CYP3A pathways was similar. Following multiple doses of EFV, the AUCs of both R- and S-5OH-OMP were decreased suggesting that the second steps in the sequential metabolism of OMP were also induced by EFV treatment. Our data show that induction by EFV enhanced the metabolism of R- and S-OMP in a non-stereoselective manner.

119. Induction of Omeprazole Metabolism by Efavirenz as a Function of *CYP2C19* Genotype

Veronique Michaud, Evan Ogburn, Nancy Thong, Abdulateef O. Aregbe, Troy C. Quigg, N Epstein, John T. Callaghan, David A. Flockhart, Zeruesenay Desta. Division of Clinical Pharmacology, School of Medicine, Indiana University, Indianapolis, IN, USA

Purpose: Efavirenz (EFV) is part of the widely used HAART regimen and drug-drug interactions associated with this drug are of major concern in the clinical setting. We have shown previously that EFV enhances the clearance of omeprazole (OMP) enantiomers in a non-stereoselective manner. OMP is extensively metabolized resulting in the formation of two major metabolites namely, 5-hydroxy-OMP (5OH-OMP) and sulfone-OMP which are produced by CYP2C19 and CYP3A4, respectively. CYP2C19 is a highly polymorphic enzyme. The aim of this study was to test whether *CYP2C19* genotypes influence the degree of induction of omeprazole (racemate and enantiomers) metabolism by efavirenz.

Methods: Healthy subjects (n=54) were administered a single dose of OMP (20 mg) after either one dose (600mg) or multiple doses of EFV (600mg/day for 17 days). Plasma concentrations of R- and S-OMP, R- and S-5OH-OMP and OMP sulfone were quantified by LC-MS/MS. CYP2C19 variant alleles (*CYP2C19*1, *2, *3* and **17*) were genotyped using Taqman assay and grouped based on predicted phenotypes as ultra-rapid (UM;*17/*17, n=4), extensive (EM;*1/*1, *1/*17, n=31), intermediate (IM;*1/*2 or*3, *2/*17, n=17) or poor metabolizer (PM;*2/*2, n=1).

Results: After a single dose of EFV, the clearance (CL) of OMP was 1.7±1.1, 0.9±0.6, 0.4±0.2 and 0.08 L/h/Kg for UM, EM, IM and PM of *CYP2C19*, respectively (ANOVA, Dunn's correction; $p<0.05$ among IM *vs* UM and EM). Following multiple doses of EFV, CL of OMP were 2.5±1.2, 1.2±0.5, 0.8±0.5 and 0.14 L/h/Kg for UM, EM, IM and PM of *CYP2C19*, respectively ($p<0.05$ IM *vs* UM groups). The same pattern of changes in clearances was observed with respect to *CYP2C19* genotypes for R- and S-isomers of OMP. After induction, no difference was observed between EM and IM groups. An inverse relationship was observed between AUC of OMP sulfone and *CYP2C19* genotypes; AUC was significantly higher in IM (2066±823 µg/L*h) compared to EM and UM groups (830±792 and 501±240 µg/L*h, respectively). A significant decrease in the metabolic ratio (MR) was observed for R- and S-5OH-OMP and OMP sulfone only in EM and IM of *CYP2C19* ($p<0.05$). The induction ratio of hydroxylation of OMP was higher in *CYP2C19* IM compared to UM group ($p<0.05$).

Conclusions: Our data show that *CYP2C19*

genotype modulates the degree of induction produced by EFV on OMP metabolism. Induction by EFV was significantly observed only in subjects with EM or IM *CYP2C19* genotypes.

120. Influence of Hyperlipidemia on the Ketoconazole-midazolam Drug-drug Interaction in Rat

Dalia A. Hamdy[1, 2] and Dion R. Brocks[1]. [1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada; [2]College of Pharmacy, Qatar University, Doha, Qatar

Purpose: Hyperlipidemia was shown to lower liver uptake of the more potent (-) enantiomer of ketoconazole in rat. This study examined the possible modifying influence of experimental hyperlipidemia on a ketoconazole pharmacokinetic interaction with midazolam.
Methods: Normolipidemic and hyperlipidemic rats were administered a single intravenous dose of midazolam 5 mg/kg with or without a single 40 mg/kg oral dose of racemic ketoconazole. Serial blood samples were collected over 8 h following midazolam injections via jugular vein cannulas. Plasma was jointly assayed for midazolam and ketoconazole concentrations using a validated assay. Student's t-test or two way ANOVA was used for statistical analysis with post hoc Bonferroni test as needed. Significance was set at $p = 0.05$.
Results: The midazolam pharmacokinetics are shown below:

	Midazolam alone		**Midazolam plus ketoconazole**	
	NL (n=6)	**HL (n=4)**	**NL (n=6)**	**HL (n=7)**
CL (L/h/kg)	2.17±0.458	2.48±0.445	1.57±0.200	1.08±0.223
Vdss (L/kg)	4.47±2.78	2.95±1.80	3.50±1.03	1.82±0.576
f_u (%)	1.97±0.38	0.760±0.29	1.78±0.15	1.02±0.21

Midazolam mean clearance (CL) was unchanged by ketoconazole co-administration. Hyperlipidemia caused a significantly 61% lower midazolam unbound fraction and decreases in volume of distribution (Vdss), but by itself had no effect on midazolam clearance. This suggested that midazolam could bind to lipoproteins. With ketoconazole coadministered to hyperlipidemic rats there were significant decreases in midazolam clearance and volume of distribution. Hyperlipidemia caused a decrease in unbound plasma fraction (f_u) of oral ketoconazole, but no significant difference in pharmacokinetic parameters.
Conclusion: Hyperlipidemia therefore resulted in a more pronounced ketoconazole-associated inhibition of midazolam clearance. This may be related to the decrease of midazolam's unbound fraction, and perhaps to the possible attenuation of CYP3A by hyperlipidemia in the rat. DAH was the recipient of a studentship from the Government of Egypt and a University of Alberta Dissertation Scholarship.

121. Dose Escalation Study of Glucosamine to Treat Adjuvant Arthritis in the Rat

Ali Agahzadeh-Habashi, Hamed Gilzad and Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Purpose: Clinical trials of glucosamine (GlcN) confirmed some positive disease modifying effect on treatment of osteoarthritis. Animal studies suggest potent anti-inflammatory effects. Nevertheless, there is still controversy regarding GlcN's effectiveness. We hypothesized that in most cases the subjects have been underdosed. Using the rat model of adjuvant arthritis, we performed a dose-effect correlation study to find out the minimum effective dose (MED) and plasma concentration of GlcN in the treatment of inflammation.
Methods: Adult male Sprague-Dawley rats were randomly assigned to six groups (n=4-5): Control-placebo, INF-placebo, INF-GlcN (20, 40, 80, and 160). On day zero INF animals received 0.2ml Mycobacterium butyricum in squalene (50 mg/mL) as tail base injection. Controls were injected normal saline. Commencing on day zero, INF-GlcN groups were orally administered GlcN HCl 20, 40, 80 or 160 mg/kg/day as aqueous solutions. Placebo received water. The Arthritis Index (AI) was assessed daily for 22 days by recording the paw and joint diameter and paw volume. The body weight of animals was also monitored. Subsequently, animals were cannulated in the right jugular veins and blood samples were collected following the last dose. GlcN in plasma was assayed using HPLC. Plasma nitrite/nitrate concentration was assessed in samples taken during canulation or right before euthanization.
Results: Animals in some of the INF groups developed arthritis in different degree based on

GlcN dose they received. Serum nitrite concentration was significantly elevated due to inflammation but GlcN treatment restored the levels. GlcN treatment showed a dose-dependent response on preventing arthritis. There was a significant correlation between AI and GlcN dose ($p<0.05$), maximum plasma concentration ($p<0.01$) and area under the concentration curve ($p<0.01$). The MED is 40 mg/kg/day that correspond to GlcN maximum plasma concentration of 1.32±0.24 mg/L in the rat.
Conclusion: GlcN has a preventive effect on adjuvant arthritis development. GlcN efficacy is dose-depended with the plasma concentration as a reliable indicator of the effectiveness.

Drug Delivery & Pharmaceutical Technology

122. Formulation Strategies to Optimize the Physiochemical Stability and Maintain the Transfection Activity of Lyophilized DNA/Gemini Surfactant Nanoparticles

Waleed Mohammed-Saeid[(1)], Deborah Michael[(1)], Ronald Verrall[(2)], Anas El-Aneed[(1)], Ildiko Badea[(1)*]. [1]Drug Design and Discovery Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK; [2]Department of Chemistry, University of Saskatchewan, Saskatoon, SK.

Purpose: DNA/cationic lipid gemini surfactant lipoplexes have been used as non-viral vectors for gene therapy. A new family of amino acid-substituted diquaternary gemini surfactants has been recently developed. The *in-vitro* transfection efficiency and toxicity profile were assessed and revealed encouraging results. However, the physical instability of the lipoplexes in aqueous formulations requires preparation of fresh nanoparticles just prior to treatment, thus limiting their potential applications. It is well established that lyophilization can retain the physiochemical properties of the nanoparticles to a certain degree. The goal of this study is to develop DNA/gemini surfactant: dioleoylphosphatidylethanolamine (DOPE) lyophilized nanoparticles using different lyophilization strategies and cryoprotectant excipients to maintain the physiochemical stability and transfection efficiency of the system.
Method and Results: The nanoparticles were prepared by using cryoprotectants such as sucrose, glucose, trehalose and lactose and additives such as glycerin, Tween 80, PEG 800 and lyophilized overnight. The freeze-dried formulations were stored at various temperatures (-20, 4, 25, 40 ^{O}C). The physiochemical characteristics (zeta-potential, size distribution, ethidium bromide accessibility and DNA integrity) and *in-vitro* transfection ability of the lyophilized formulations were evaluated. In addition, a multiple reaction monitoring-Liquid Chromatography/ Mass spectrometry (MRM-LC/MSMS) method was developed for the purpose of quantifying the gemini surfactant used in the lyophilized formulations and to study its degradation products. Our results showed that most of the nanoparticles lost their transfection efficiency due to the disassembling of the DNA/gemini surfactant/DOPE complex and DNA degradation. However, lipoplexes formulated with 10% Tween 80 (w/w) and 9.25 % sucrose (w/w) retained the original physiochemical properties of the freshly prepared formulations.
Conclusion: This formulation will be used in the future for systematic evaluation of the stability using International Conformance of Harmonization (ICH) guidelines for accelerated stability study.

123. Reactivity of Prednisolone to Gamma Radiation in Aqueous and Organic Solutions

Ahmed Bosela[1], Khloud Salamah[1], Ibrahim Alsarra[1, 2], Ibrahim El-Bagory[1]. [1]Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia [2]Center of Excellence in Biotechnology Research, King Saud University, Riyadh, Saudi Arabia

Purpose: The purpose of the present study is to investigate the destructive effect of radiolytic products of solvent, either aqueous or organic, on the drug during gamma irradiation and the kinetics of such degradation. The protective effect of different types of free radical scavengers as well as surfactants, anionic, cationic and nonionic will be also studied.
Methods: The reactivity of prednisolone (Pd) to gamma radiation in aqueous and organic solutions has been investigated using Cobalt 60 source in a Gammacell-220 and radiation doses of 0.25, 0.5, 1.0, 2.0, 3.0, 4.5, 6 and 9 KGy. The residual drug concentration was determined by HPLC method

using 45:55 acetonitrile: water as mobile phase on a reversed phase column and UV detector at 254 nm.
Results: The degradation kinetics study showed an initial zero-order followed by complex reactions illustrated graphically as an overall first order kinetic. The study revealed that the drug was significantly more sensitive to gamma radiation in aqueous solutions than organic solvents, with degradation rates of 1.34 and 0.67 x 10-4 M/KGy for aqueous and propylene glycol solutions, respectively. The structure activity relationship of the organic solvents (ethanol, n-propanol, isopropanol, n-butanol, isobutanol, tertiary butanol, 1-3 propane-diol, propylene glycol and glycerin), as pure solvents has been studied. Although the difference was not significant, the alkyl chain length and branching increased the reactivity of the solvent to radiation. Moreover, the number and position of hydroxyl groups in the solvent structure have significantly increased its sensitivity to radiation which has been manifested as increased drug degradation. Isoropanol and tertiary butanol as free radical scavengers have shown variable protective effects for the drug against radiation.
Conclusion: The role of different types of surfactants (sodium lauryl sulfate, Tween 80 and benzalkonium chloride) in drug protection against radiation in aqueous solutions suggested two different mechanisms. The first is the scavenging effect of the surfactant monomers below its CMC. Above CMC, a second mechanism may be involved, where the drug is expected to be located inside the formed micelles to different extents according to its affinity to the surfactant leading to more stabilization of the drug. The protective effect of surfactants was found to be in the order: sodium lauryl sulfate > Tween 80 ≥ benzalkonium chloride.

124. Alternate Method for Calculating Density Factor of Cocoa Butter used as a Standard

Dr. Kenneth S. Alexander, Heather Walker, Vishak Sridhar. College of Pharmacy, Industrial Pharmacy Division, The University of Toledo, Toledo OH

Background: The cocoa tree, which produces cocoa beans, exists in nature as three different varieties: Criyollo, Forastero and Trinitario. This variety of tree grows in limited geographical zones around the world. The cocoa butter which is extracted from these different geographical regions contains different percent compositions of fatty acids, namely different ratios of palmitic acid, stearic acid, oleic acid, and linoleic acid. These in turn affect the density of the cocoa butter. The density of cocoa butter is a key factor in calculating the weight of cocoa butter required for making suppositories. There are three methods used to determine the amount of base needed in preparing suppositories. These methods will be described and a new method proposed. The density of the cocoa butter is found to be from 0.86-0.94, or as given in the USP/NF as 0.9 which is an average value.
Experimental Methods: The composition of cocoa butter fatty acids, palmitic acid, stearic acid, oleic acid, and linoleic acid, from the literature and other sources were compared. The physical properties of the cocoa butter from these different sources were evaluated and the density of each was determined. The density factors for various drugs were estimated by an empirical method which used the densities and percentages of the individual fatty acid components to determine the density of the cocoa butter.
Results and Discussions: The composition of the fatty acids contributed to the density of the cocoa butter which varied from source to source. The density was determined by using the densities and percentages of the individual fatty acid components of the cocoa butter. Every source had a different density, which fell in a range based on composition. Since the density varied from source to source, an average value of 0.9 would not be an appropriate value to use when completing suppository base calculations for cocoa butter.
Conclusions: The density for cocoa butter will be diverse based on the origin of the source. By using the actual fatty acid composition, a more reasonable density of the cocoa butter could be calculated. This value was then used to determine the density factor for a number of drugs which uses cocoa butter as the standard. This new method, using the actual density for the cocoa butter, rather than using the reported average value of 0.9 in the standard calculations, will allow for a more accurate value for determining the cocoa butter density factor for any new drug which might be compounded or manufactured as a suppository dosage form.

125. A Study of the Sedimentation Characteristics of Never Dried Magnesium Hydroxide Suspensions

Dr. Kenneth S. Alexander, Sriramya Punnamaraju. College of Pharmacy, Industrial Pharmacy Division,

The University of Toledo, Toledo OH.

Background: This project focuses on the preparation and characterization of never dried magnesium hydroxide suspensions produced from epsom salt (magnesium sulfate heptahydrate) and sodium hydroxide. Never dried suspensions have many advantages over suspensions manufactured from the dried powders. They do not sediment rapidly, are easily redispersible and have fewer problems compared to dried magnesium hydroxide dispersed directly in the medium.
Experimental Methods: Various quantities of powder magnesium hydroxide (1, 3, 5, 7 and 9 Gms) were prepared stoichiometrically by adding sodium hydroxide solution to Epsom salt in a 250 mL graduated cylinder and left undisturbed for 24 hours. The supernatant is decanted and made up to 250ml with purified water. The same process is repeated till the suspension is free of sodium sulfate ions. The suspension was then finally made to 250 ml and change in the height of the interface was recorded with respect to time. This process was repeated using different concentrations of Poloxamer (0.005, 0.01 and 0.02%) as the dispersion medium for magnesium hydroxide. The effect of the polymers in the dispersion medium was evaluated using the hindered settling theory, SEM and Laser Diffraction.
Results and Discussion: The rate of fall was calculated by plotting interface height versus time. The effect of dispersion media on the particle size was investigated using the Richardson & Zaki, Steinour, and Dollimore & McBride equations. In addition, the Kozeny-Carman equation which considers permeability is also used to determine particle size of the suspensions The hindered settling theory showed that Poloxamer stabilized magnesium hydroxide suspensions with a higher rate of fall of particles, increasing viscosity, and a slightly enlarged particle size. The initial radius for magneisum hydroxide was 5.65 μm, and increased with increasing concentrations of Poloxamer to a maxium of 8.49 μm.Particle size distribution is determined by laser diffraction and technique.SEM studies indicate the presence of rod shaped crystals of Magnesium hydroxide which appeared in the form of floccules.
Conclusions: Results indicate the stabilization of suspensions by the non ionic polymer, Poloxamer. There is good correlation between the particle size determined from the hindered settling theory and SEM studies. Poloxamer when used in lower concentrations caused the flocculation of the suspension but higher concentrations cause the suspension to deflocculate.

126. Carboxymethyl Starch Microspheres as Mucoadhesive Dosage Form

Marc Lemieux[1], Patrick Gosselin[2], Joanne Paquin[1], Mircea Alexandru Mateescu[1,*]. [1]Dept. of Chemistry and Centre Pharmaqam, Universite du Quebec a Montreal, Montreal, QC; [2]Corealis Pharma Inc, Laval, QC. * Corresponding author

Purpose: Develop microspheres using carboxy-methyl high amylose starch (CMS) with different degrees of substitution (DS) and evaluate their physicochemical, dissolution and mucoadhesive properties as well as their interactions with gastrointestinal epithelial barrier models for the oral delivery of small molecules.
Methods: The microspheres (90 mm diameter) were produced from CMS with a degree of substitution varying from 0.1 to 1.5 by emulsion cross-linking, and loaded or not with furosemide (10% w/w) as drug model. The drug delivery properties of the CMS microspheres were determined by dissolution tests in pH 1.0 and 7.4 buffers. Their action on cells was evaluated with the human epithelial cell lines NCI-N87 (gastric) and Caco-2 (intestinal) cultured as monolayers on a porous support. The cultures were analysed for their transepithelial electrical resistance (TEER) and the permeability coefficient (P_{app}) of furosemide at the following pH values: 7.4 on basolateral and apical sides (Caco-2); 7.4 basolateral and 3.0 to 7.4 apical (NCI-N87). The cytotoxicity of the CMS microspheres was evaluated with the tetrazolium salt (XTT) viability assay. Their mucoadhesive property was assessed with wash-off tests using sections of porcine gastric and intestinal mucosae.
Results: The dissolution rate of furosemide from the CMS microspheres in pH 1.0 buffer decreased with DS while in pH 7.4 buffer, the furosemide release was immediate. Furthermore, the loading of furosemide into the CMS microspheres increased its solubility at pH 1.0 from 5 to 30 mg/ml compared to pure API. The TEER of NCI-N87 monolayers was not influenced by the presence of CMS microspheres at their surface, whereas that of Caco-2 monolayers decreased but recovered initial values within 15 h post-treatment. The CMS microspheres also enhanced the permeability of furosemide across the NCI-N87 and Caco-2 monolayers at all pH. The cell

viability test indicated that these microspheres were not cytotoxic up to 10 mg/ml at all DS. Mucoadhesion of the CMS microspheres on gastric mucosa (acidic condition) increased with DS up to a DS of 1.0 but decreased with DS on intestinal mucosa (neutral condition).
Conclusion: CMS with DS between 0.6 and 1.0 are suitable for the formulation of gastroretentive mucoadhesive microspheres.

A poster covering in part this work was presented at the 2010 FIP PSWC/AAPS Annual Meeting and Exposition (November 13-18, New Orleans, LA) and for which one-page abstract was published in The AAPS Journal, 2010 12(S2) (Available from: http://www.aapsj.org/).

127. The Development and Manufacture of Thermal Stable Vaccines

Ron Boch, David Anderson, Jasminka Bozic, Marc Kirchmeier, Alexandre Lavigne, Turaya Naas, Andrei Ogrel, Svetlana Ogrel, Barthelemy Ontsouka. VBI Inc., Cambridge, MA; Ottawa, Ontario

Purpose: The total established vaccine market is expected to exceed $21 billion in 2010 and 97% of all vaccines (or $20.6 billion of that market) require "cold chain" shipment at 4 degrees Celsius. Without a constant temperature in a very narrow range above freezing, many currently available vaccines lose their potency, become ineffective or can become hazardous. It is estimated that this cold chain actually increases vaccine costs 14-20%. The purpose of this Study is to develop and manufacture a thermal stable formulation capable of preserving hemagglutinin protein based influenza flu vaccine.
Methods: Formulations of thermal stable vaccines were prepared with substances with well known safety profiles in humans and existing, off-the-shelf or novel vaccine antigens. Non-adjuvanted and adjuvanted vaccine formulations were prepared and scaled up to batch sizes required for clinical evaluation.
Results: Thermostability was demonstrated preclinically (*in vitro* and *in vivo)* at 4°C, 25°C, and 40°C for extended periods of time up to 6 months. Efficacy and immunogenicity of VBI formulations were further confirmed in ferrets and rhesus macaques.
Conclusion: A proprietary thermal stable intramuscular product that can withstand a full 12 months of storage or shipment at elevated temperatures as high as 40º C was developed and prepared and scaled up toward increased safety, efficacy and enable expanded vaccine access to established and emerging markets.

128. Identification and Quantitation of Gemini Surfactant Nanoparticle Constituents by Simple Direct Mass Spectrometric Infusion and Conventional Liquid Chromatography Electrospray Ionization Mass Spectrometry

Joshua Buse[1], Waleed Mohammed-Saeid[1], Deborah Michel[1], Ildiko Badea[1], Ronald E. Verrall[2], and Anas El-Aneed[1*]. [1] Drug Design and Discovery Research Group, College of Pharmacy and Nutrition, University of Saskatchewan; [2] Department of Chemistry, University of Saskatchewan, Saskatoon, SK

Purpose: Gemini surfactant nanoparticles have been extensively studied as non-viral vectors for gene therapy. The general molecular structure of the gemini surfactants evaluated in this study consists of two symmetrical hydrophobic tails attached to two positively charged quaternary ammonium head groups that are linked via a spacer region. The spacer can contain various functional groups, including a subgroup that is substituted with amino acid moieties. The purpose of this study is to develop mass spectrometric (MS)-based analytical detection methods of these vectors which will allow for the qualitative and quantitative analysis of these compounds within various matrices.
Methods: An Applied Biosystems, API QSTAR XL hybrid tandem mass spectrometer (MS/MS) was utilized for MS/MS analysis with electrospray ionization (ESI). For quantitation purposes, two lead compounds were selected to develop MS-based quantification methods. One compound contained a short hydrophobic spacer region with C16 saturated alkyl tails while the second gemini surfactant was comprised of a glycine-substituted spacer along with C12 saturated alkyl tails. An AB Sciex API 4000 Q-Trap mass spectrometer coupled to an Agilent 1100 Binary Pump and Autosampler modules was used. A gradient was utilized to elute tested compounds using C18 and cyano columns.
Results: MS/MS analsyis of 35 novel compounds resulted in the development of a universal fragmentation pattern (i.e. MS/MS fingerprint). MS/MS fingerprints allowed for the qualitative identification of the tested compounds within a

formulation or tissue culture matrix.
Preliminary quantitative data demonstrated that the use of multiple reaction monitoring (MRM)-MS/MS quantitation of the glycine-substituted gemini surfactant resulted in comparable selectivity and sensitivity using both direct MS infusion or HPLC-MS/MS. Direct infusion is superior to LC-MS/MS; it is less time consuming with significant reduction in the use of solvents. Similarly, the lead gemini surfactant that bears no substituents in the hydrophobic spacer was successfully quantified using HPLC-MRM-MS/MS within a tissue culture matrix, achieving a limit of quantitation of 3 μM. Preliminary data indicates that direct MS infusion within the tissue culture matrix was feasible, achieving higher sensitivity than conventional HPLC-MRM-MS/MS method.
Conclusion: Mass spectrometry can be effectively used to identify and quantify gemini surfactants within various matrices. The compounds of interest were successfully quantified within a formulation or tissue culture matrix. The developed quantification methods will be employed to assess the stability of novel gemini surfactant-based delivery systems and to monitor their cellular fate following transfection.

129. PGD_2-loaded Microspheres as Therapeutic Strategy for the Treatment of Histoplamosis

Priscilla A. T. Pereira[1], Claudia S. Bitencourt[1], Guilherme M. Gelfuso[1], Daiane. F. dos Santos[1], Adriana Secatto[1], Ruxana T. Sadikot[2], Lúcia H. Faccioli[1]. [1]Análises Clinicas, Toxicológicas e Bromatólogicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Ribeirão Preto, Brazil; [2]Section of Pulmonary, Critical Care and Sleep Medicine, University of Illinois, Chicago, USA.

Introduction: PGD_2 and PGE_2 play an important role in inflammatory processes, stimulating or inhibiting the removal of microorganisms in the host, respectively. However, these compounds possess poor hydrosolubility and high chemical instability, not allowing the *in vivo* administration. Microspheres (MS) improve stability and sustain the release of substances and target their deliveries. Purpose: the aim of this work was to develop MS containing PGD_2 and evaluate their role in histoplasmosis.
Methods and Results: PLGA [poly-(lactic acidglycolic acid)] (50:50) MS were prepared by the emulsification-solvent evaporation technique. Size distribution and zeta potential were evaluated in aqueous media by Light Scattering (LS), and morphology analyzed by Scan Electronic Microscopy (SEM). These analyses revealed that all the microspheres presented spherical shape, with no pores on their surfaces, mean size equal to 4.0 (± 2.5) μm (MS-loaded-PGD_2) and 3.7 (± 2.1) μm (MS-loaded-PGE_2, used as control). The zeta potential was negative for MS-loaded-PGD_2 (-10.1 ± 7.9 mV) and MS-loaded-PGE_2 (-13.7 ± 5.7 mV). All the preparations were proper to be administered through the intranasal route and able to reach the lung. *In vitro* MS phagocytosis was determined after 4, 8, 24 or 48 hours of incubation with 1 mg/mL of each MS using 2 x 10^5 cell/well of rat alveolar macrophages (AMs). Both MS were efficiently phagocyted by cells. After 4 and 48 hours of incubation, MS-PGD_2 had the highest phagocytic index and MS-PGE_2 the lowest, when compared with unloaded-MS. MS-PGD_2 induced NO_2^- production by AMs. In C57Bl/6 mice infected intratracheally with *Histoplasma capsulatum,* the intranasal treatment with 4 doses of MS-PGD_2 decreased cell recruitment to the bronchoalveolar space and decreased the number of yeasts (CFU) in the lung.
Conclusion: MS obtained in this work could be an novel approach to the development of therapeutics based on lipid mediators, and which afford protection against *H. capsulatum* infection.

130. Drug-induced Protein Free Radical Formation is Attenuated by Linoleic Acid by Scavenging Drug-derived Phenyl Radical Metabolites

Malyaj Narwaley, Pratik Arvadia, Arno G. Siraki. University of Alberta, Faculty of Pharmacy & Pharmaceutical Sciences, Edmonton, Canada.

Aim: My aim is to detect phenyl radical metabolite formed due to peroxidase metabolism of aromatic amine drugs and to find their reactivity towards unsaturated intra cellular lipids as potential mechanistic approach for their toxicity.
Method: Aminoglutethimide, 4- chloroaniline and aniline were taken as a model drugs for the phenyl radical generation due to peroxidase (HRP)/H_2O_2 metabolism. Linoleic acid (LA) was selected as a target unsaturated lipid molecule for the

metabolically generated phenyl radical as mentioned above. Other related aromatic amines and unsaturated lipids were also tested for the reactivity between the phenyl radical and unsaturated lipids. The phenyl radical was detected in ESR and its reactivity towards unsaturated lipids was tested using Clark type oxygen electrode, ESR, and western blot immunoassay.

Results and Conclusion: O_2 uptake was observed in oxygen electrode by aromatic amine drugs, in the presence of LA, HRP and H_2O_2, in phosphate buffer. No O_2 uptake was observed in the absence of one reactant, suggesting an oxidative reaction between the phenyl radical metabolite and LA occurred, resulting in lipid peroxidation. Further, ESR studies confirmed attenuation of phenyl radical metabolites in the presence of HRP/H_2O_2 by LA. Western blot analysis confirmed the attenuation of phenyl radical by linoleic acid which resulted in inhibition of phenyl radical mediated myeloperoxidase radical formation in HL-60 cells. The oxidation of unsaturated lipids by phenyl radical metabolites of drugs represents a possible pathways in the toxicological side effects for certain drugs.

131. Artificial Cell APA Microcapsules for the Delivery of a *Lactobacillus fermentum* Based Therapeutic

Catherine Tomaro-Duchesneau, Shyamali Saha, Meenakshi Malhotra, Hani Al-Salami, Imen Kahouli, Satya Prakash*. Biomedical Technology and Cell Therapy Research Laboratory, Departments of Biomedical Engineering, Physiology, and Artificial Cells and Organs Research Center, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

Purpose: Alginate-polylysine-alginate (APA) microcapsules can be used for the delivery of probiotic bacterial cells. Research has identified low-grade inflammation as an important contributing factor to the development of a large number of disorders. The data has turned to the gut microbiota, the "microbial organ" as an important link between low-grade inflammation, high-fat foods and metabolic disorders, suggesting that prebiotics, probiotics, and synbiotics could be used efficiently as therapeutics to increase the number of beneficial bacteria, decreasing the prevalence of low-grade inflammation. *Lactobacillus fermentum* has demonstrated characteristics beneficial to human health, with the enzymatic activity of ferulic acid esterase. One main obstacle of probiotic delivery is the harsh environment of the human gastrointestinal tract which is detrimental to the delivery of cells, quickly leading to a significant decrease in cell viability. Microencapsulation, specifically APA microcapsules, has been developed as a delivery mechanism to overcome this difficulty.

Methods: APA microcapsules were designed, prepared and characterized in-vitro for bacterial cell oral delivery using *L. fermentum* cells and an Inotech microencapsulator. Microcapsule structural integrity and gastrointestinal studies were performed in simulated gastric (SGF) and intestinal (SIF) fluids in various pH conditions at 37°C, and results were compared to free cells. Mechanical stability was determined by microscopy, viability by colony counting methods and enzymatic activity by HPLC.

Results: 300µm APA microcapsules containing *L. fermentum* cells remained mechanically stable following exposure to SGF for a period of 2 hours and following exposure to SIF for 8 hours, as determined by microscopy. The viability of the encapsulated *L. fermentum* was 2 logs higher (with an initial viability of 10^9 cfu/mL) than free bacterial cells, following exposure to the simulated gastrointestinal conditions. The enzyme activity, specifically ferulic acid esterase was also maintained at a higher activity in the encapsulated bacteria when compared to the non-encapsulated.

Conclusion: This APA microcapsule provides a barrier to the harsh conditions of the GIT while allowing for the flow of cellular waste products out of the microcapsule so that the encapsulated cells can continue to be metabolically active. The use of microcapsules can only increase the beneficial effects of probiotics, specifically that of *L.fermentum* and could be combined with a prebiotic for a synbiotic microencapsulated formulation.

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132. Qualification of MFI for Sub-visible Particle Analysis in Protein Formulations

D. Sharma[1], C. Merchant[1], D. Thomas[1], D. King[1]. [1]Brightwell Technologies Inc., Ottawa, Canada

Purpose: Sub-visible particles in 2 to 10µm size range that are often present in protein therapeutics have been identified by the regulatory agencies as a

potential safety issue. These particles can be highly transparent, fragile, unstable and difficult to detect using conventional light obscuration based methods. The need has therefore been identified for new analytical methods which can accurately measure these particle types. These analytical methods are required to be sufficiently quantitative and robust as to allow application from R&D formulation development through to QC lot-release testing. This paper will describe the qualification of an MFI method to characterize the particle size and concentration levels of sub-visible particulates present in therapeutic protein formulations. Case-Studies will be presented, illustrating successful application of the ICH guidelines for analytical method qualification and validation.

Methods: Sizing, counting accuracy and precision of particle size determinations were performed by the MFI method using certified PS beads of known size and quantity. Instrument counting and method precision of particle measurements by the MFI method was performed using proteinaceous particles from a pool of vials containing a 90 mg/mL mAb formulation stored for 18 months at 2-8°C.

Results: MFI was found to have similar detection sensitivity to light obscuration for PS size standards, however for proteinaceous samples MFI was found to be significantly more sensitive. In some cases an order of magnitude or more particles were detected, particularly in the 2 to 10µm size range. MFI counting and sizing was found to be highly accurate and precise for both PS size/concentration standards and proteinaceous particles. Very low RSD's were achieved, in consideration of test repetitions, operators, instruments, and time. MFI showed excellent linearity for quantifying proteinaceous particles in opalescent solutions over a wide range of particle concentrations.

Conclusion: An MFI assay was qualified as a method to count and monitor protein particle size and quantity in a high concentration monoclonal antibody formulation (in the subvisible and visible size range).

133. Biodistribution and Toxicity of Amphotericin B in Mice Following Multiple Dose Administration of a Novel Oral Lipid-based Formulation (iCo-009) and Water-based Suspension of Amphotericin B

Pavel Gershkovich[1,*], Olena Sivak[1], Ellen K. Wasan[1,2], Alex B Magil[3], David Owen[4], John G. Clement[5], and Kishor M. Wasan[1]. Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC.

Purpose: To assess the biodistribution and toxicity of amphotericin B (AmB) following multiple dose administrations of either iCo-009 (a novel oral lipid-based formulation of AmB) or a water-based suspension of AmB

Methods: BALB/c female mice were allocated into the following treatment groups: oral gavages of iCo-009 twice daily for 5 days at doses of either 20 mg/kg, 10 mg/kg, 5 mg/kg or 2.5 mg/kg; oral gavages of vehicle control (170µl, twice daily for 5 days); oral gavages of water-based suspension of AmB twice daily for 5 days at doses of either 10 mg/kg, 5 mg/kg or 2.5 mg/kg; and intravenous (IV) boluses of Fungizone® (as a positive control of kidney toxicity only) once daily for 5 days at doses of either 1mg/kg (n=9) or 2mg/kg (n=10). The animals were sacrificed 12h following the last administration of AmB and blood and multiple tissues were harvested for drug analysis of the oral formulations only by HPLC-UV and histopathological evaluation. Creatinine concentrations in plasma were determined by HPLC-UV.

Results: The multiple dose administration of iCo-009 has resulted in a dose-dependent accumulation of AmB in liver, spleen and lung tissues with a concentration of the drug in all these organs being as high as ~5µg/g for 20mg/kg dose, exceeding the corresponding concentrations in plasma. The levels of AmB in heart and brain were similar to the corresponding concentrations in plasma. The administration of water suspension resulted in dramatically lower (compared to iCo-009) concentrations of amphotericin B in all tested organs, especially in reticulo-endothelial system. Higher volume of administered lipids corresponded with more dramatic differences between iCo-009 and water suspension. The creatinine levels were in a normal range, except the Fungizone® 2mg/kg group in which the creatinine concentration reached levels consistent with moderate renal toxicity (~0.28 mg/dl). The histopathology analysis of kidney and liver tissue revealed a non-toxic pattern, except the Fungizone® 2mg/kg group which showed tubular epithelial necrosis (kidney) or necrotic hepatocytes (liver). No gastrointestinal toxicity was found in this study.

Conclusions: A multiple dose treatment regimen with iCo-009 in mice resulted in a gradual

accumulation of AmB in tissues without any signs of liver, kidney or gastrointestinal toxicity. The data suggests that lipid component of iCo-009 formulation significantly facilitates the intestinal absorption of AmB relative to water suspension.
Funding: CIHR and iCo Therapeutics Inc.

134. Tri Block Copolymers of Poly (ethylene glycol) and Functionalized Poly(ε-caprolactone): Synthesis and Characterization of their Thermo/pH Responsive Assembly

Nazila Safaei Nikouei[1] and Afsaneh Lavasanifar[1, 2*]. [1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta; [2]Department of Chemical and Material Engineering, University of Alberta, Edmonton, Alberta, Canada

Purpose: The aim of this study was to synthesize tri block copolymers based on PEG and α-carbon functionalized poly(ε-caprolacton) and assess the potential of these ABCs in thermo and/or pH responsive gel formation through characterization of their stimuli responsive micellar assembly.
Method: A series of novel tri block copolymer composed of poly(ethylene glycol) (PEG) in the middle and poly(ε-caprolactone) modified with benzyl carboxylate groups on the α-carbon of ε-caprolatone were synthesized through ring opening polymerization of α-benzyl carboxylate-ε-caprolactone by dihydroxylated PEG. The debenzylation of synthesized copolymer, i.e., poly(α-benzyl carboxylate-ε-caprolactone)-*b*-PEG-*b*-poly(α-benzyl-carboxylate-ε-caprolactone) (PBCL-*b*-PEG-*b*-PBCL), in the presence of hydrogen gas was carried out to achieve copolymers with various degrees of free α-carboxyl to α-benzyl-ε-carboxylate groups on the hydrophobic block. Incomplete reduction of PBCL led to the formation of poly (α-carboxyl-co-benzyl caboxylate-ε-caprolactone) PCBCL in the lateral blocks. The molecular weight of the resultant copolymers was determined by ^{1}H NMR and MALDI. The size of polymeric micelles was assessed by dynamic light scattering, where the change in micellar size for aqueous solutions of different copolymers was measured as a function of temperature and pH.
Result: The presence of carcoxylic group on PCBCL chain was shown to introduce pH as well as thermo sensitivity to the assembly of the micellar structure. At neutral pH, polymers with 27 % degree of debenzylation illustrated a sharp rise in micellar size while polymers with higher degrees of debenzylation showed a decrease in micellar size as a function of temperature. All block copolymers under study showed a decrease in micellar size at pH ≥4.5, due to unionization of carboxyl group which will make the PCBCL block more hydrophobic.
Conclusion: The results points to a potential of pH tri block copolymers of PCBCL-*b*-PEG-*b*-PCBCL in the formation of thermo- and pH responsive biodegradable gels.

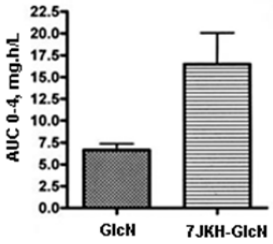
135. From Nutraceutical to Pharmaceutical: A Novel Peptide Pro-drug of Glucosamine with Increased Bioavailability

Mohammadhossein Gilzad Kohan, Kamaljit Kaur, and Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Purpose: Glucosamine (GlcN) is a naturally occurring amino sugar with anti-inflammatory effects which is vastly administered to treat osteoarthritis; however its poor bioavailability (F = 0.11 - 0.19), its status as a non-regulated marketed compounds, and the need for high doses to exert therapeutic effects, limit its benefits. The aim of this study was to synthesize pro-drugs of GlcN with increased bioavailability through the peptide transport system, more specifically, the peptide transporter 1 (PEPT-1).
Methods: Using a solid-phase synthesis approach, on an appropriate resin, several GlcN ester and amide derivatives were synthesised. Briefly GlcN was dissolved in a suitable solvent and added to the pre-swelled resin. Coupling of the resin and GlcN was achieved by stirring the mixture overnight at room temperature. The resin was drained and washed to remove the unreacted reagents. Subsequently, the Fmoc or Boc protected amino acids were activated by BOP, HOBt, and NMM in DMF. The activated amino acid was added to the resin and the mixture was stirred for several hours at room temperature. After draining and washing, Fmoc protecting group was removed by piperidine 20% in DMF prior to final cleavage. The Boc removal was achieved during the final cleavage step. In vitro absorptions of the new pro-drugs through everted rat gut were assessed; compounds with increased absorption were picked to be tested for their efficiency to biotransform to GlcN in the liver

and in vivo bioavailability in the rat. Plasma GlcN concentrations in serial sample were measured using HPLC.

Results: among 16 different pro-drug containing various amino acids and different sequences, only one, 7JKH-GlcN, demonstrated significant and meaningful increased bioavailability; three fold increased area under plasma GlcN concentration-time (AUC0-4) as compared with GlcN HCl .



Other compounds failed due to a lack of stability in the gut, negligible penetration through the gut and/or insufficient conversion to the parent compound. This successful compound showed acceptable stability in the gut and a quick cleavage to GlcN after exposure to liver homogenate.

Conclusion: A novel derivative of glucosamine with superior bioavailability was developed and successfully tested in vivo and in vitro.

136. Design, Development and Evaluation of Novel Nanoemulsion for Improved Bioavailability of Simvastatin

Sandip Chavhan, Kailash Petkar, and Krutika Sawant. Drug Delivery Laboratory, TIFAC CORE in NDDS, Pharmacy Department, The M S University of Baroda, Fatehgunj, Vadodara, Gujarat, India.

Purpose: Simvastatin is poorly bioavailable as it is practically insoluble in water and shows dissolution rate-limited absorption. Therefore, the aim of the present study was to prepare novel nanoemulsion of simvastatin for improving its solubility and/or dissolution rate which may lead to enhancement in its bioavailability.

Method: Novel nanoemulsion was prepared by ultrsonic emulsification method. The selection of the oil was based on solubility of the drug while surfactants were selected based on stability of the prepared nanoemulsion. Formulation parameters were optimized using factorial design. The nanoemulsions were evaluated for particle size, zeta potential, TEM, viscosity, *in-vitro* release and stability studies. The *in vivo* pharmacodynamic studies were conducted in hyperlipidemic rats and reduction in total cholesterol and triglyceride level was used as basis for the comparison of *in vivo* performance of the nanoemulsion and plain drug.

Results: The parameters (oil concentration and ratio of hydrophilic to lipophilic surfactant) which have major effect on particle size and stability were considered for factorial design. The optimized nanoemulsion showed particle size of 132±9nm while zeta potential was found to be 17.1±1.2 mV. TEM studies confirmed particle size of the globules and showed spherical globules. *In-vitro* release studies showed increased dissolution rate of the nanoemulsion as compared to plain drug. Nanoemulsion was found to be stable for 3 months at room temperature and cold conditions. Pharmacodynamic studies in rats showed significant decrease in the total cholesterol, triglyceride levels for nanoemulsion as compared to plain drug indicating improvement in bioavailability.

Conclusion: The novel nanoemulsion of simvastatin prepared by ultrasonic emulsification method significantly improved its solubility and dissolution rate which in turn improved absorption and bioavailability that was reflected in significant hypolipidemic activity. Thus, it can be concluded that nanoemulsion have tremendous potential for improving bioavailability of poorly water soluble drugs like simvastatin.

Keywords: Nanoemulsion, factorial design, bioavailability, *in vitro* release, hypolipidemic activity.

137. Lipogel of an Anti-inflammatory Agent: Randomized, Double-blind Clinical Trial for Evaluation of the Efficacy and Safety in Patients with Signs and Symptoms of Osteoarthritis of the Hip, Knees and Hands

Vijay G Goni[1], Kaisar Raza[2], Amit Bhatia[2], Pebam Sudesh[1], Nirmal Raj Gopinathan[1], Shakthi Vel Rajan[1], Shashidhar B. Kantharajanna[1], Bhupinder Singh[2], O P Katare[2]. [1] Department of Orthopaedic Surgery, Post Graduate Institute of Medical Education and Research, Chandigarh; [2]Drug Delivery Research Group, University Institute of Pharmaceutical Sciences – UGC Centre for Advance Study, Panjab University, Chandigarh, India.

Purpose: To compare topical etodolac in

deformable-liposomal gel *versus* marketed gel and placebo for relief of signs and symptoms in osteoarthritis (OA) of knee.

Methods: This was a randomized, double-blind, controlled trial on 36 patients with knee osteoarthritis. They were randomly assigned to flexible-liposomal formulation, active marketed formulation and placebo, three times a day for 6 weeks. The patients were assessed by primary efficacy outcome measures included the changes from baseline to end of study on the WOMAC (Western Ontario McMaster Universities) Osteoarthritis Index. The radiographic grading of OA in the knee was performed by using the Kellgren–Lawrence criteria. We also assessed the safety by evaluation of adverse events, vital signs and irritation at the application site.

Results: In flexible-liposomal gel group the pain, stiffness and difficulty performing routine activities showed statistically significantly improvements on 6 weeks of treatment compared to the other tested formulations. All the treatments were found to be well tolerated with no adverse effects.

Conclusion: Etodolac in deformable-liposomal gel was found to be superior to other tested formulations *viz* marketed gel and placebo in the relieving the symptoms of OA of the knee. Hence, it can be concluded that etodolac in flexible-liposomal gel can be a rational alternative to oral etodolac formulations for management of various pain and inflammation related ailments including osteoarthritis.

CSPT Posters - Day 2

Thursday, May 26, 2011

138. Calcium Involved in the Vasorelaxant Effect of Convolvulus Arvensis L Extract on Rabbit Aorta Rings

Al-Aghawani W, Abu Jayyab A. Faculty of Pharmacy, International University for Science & Technology, Daara, Syria
Corresponding Author: jayyab@yahoo.com
Conflict of Interest: None declared

Background: Our previous finding suggests that calcium plays an important role in the relaxant effect of *Convolvulus arvensis* L (CAL) extract in rabbit's aorta.
Objective: As a result, further investigation is needed to determine calcium role in the CAL induce relaxation.
Methods: CAL extract was added cumulatively to the rabbit aorta pre-contracted with high K^+ Krebs solution. Also another procedure was performed using Ca^{2+}-free Krebs where aorta ring exposed to Phenylephrine (PE) as a control group. When the phasic contractions reached a plateau, $CaCl_2$ was added to the bath, causing a tonic contraction. This procedure effectively explain whether the relaxant action of CAL was due to intracellular Ca release from the endoplasmic reticulum (ER)or extracellular Ca^{2+}-release from cell surface calcium channels. The procedure was repeated in the same tissue after pretreatment of extract or Diltiazem.
Results: CAL significantly decreased vessels contraction induced by high concentration of K^+. Also, the CAL extract inhibited both calcium release from the ER and the entry of calcium through Ca^{2+} channels.
Conclusions: Our finding gives evidence that clearly CAL induced relaxation in rabbit Aorta rings via involvement in the mobilization of Ca^{2+} from ER as well as from Ca^{+2} channels. Our results might led to new therapeutic application of CAL in cardiovascular diseases.

139. Bone Marrow Cells Migrate to the Retina and Effect Angiogenesis in a Mouse Model of Oxygen Induced Retinopathy

Blais M[1], Rivera JC[1], Madaan A[1], Sapieha M [3], Chemtob S[1,2]. [1]Research Center, Sainte-Justine Hospital, Montreal, Quebec, Canada; [2] Department of Ophthalmology and Pharmacology, University of Montreal, Montreal, Quebec, Canada; [3] Department of Ophthalmology, Maisonneuve-Rosemont Hospital Research Centre, University of Montreal, Montreal, Quebec, Canada
Corresponding Author: martineblais@rogers.com
Conflict of Interest: None declared

Background: The retina is a metabolically active tissue requiring a large amount of oxygen. Under hypoxic conditions, neovascularization occurs and can have severe negative repercussions on the retina. Retinopathy of prematurity (ROP), the leading cause of infant blindness, exemplifies the detrimental effects neovascularization can cause. These premature newborns acquire vaso-obliteration of micro vessels in the retina, followed by a pathological neovascularization once the retinal metabolic demand increases. Oxygen induced retinopathy (OIR) is an animal model that replicates both phases of ROP and is achieved by exposing newborn mouse pups to 80% oxygen from post-natal day 7 to 12. There have been many advances in stem cell research regarding coronary and renal revascularization demonstrating a possible role for stem cells in angiogenesis.
Objective: Utilizing bone marrow derived stem cells, we aimed to repopulate the retina with normal vessels which are affected in the OIR model.
Methods: Two different cell types were isolated from mouse bone marrow: lineage negative (Lin-) and mesenchymal stem cells (MSC). These cells were then injected into the vitreous of OIR mice.
Results: Mouse retinas collected at P17 from both MSC and Lin- injected mice demonstrated cell migration to the inner retina. Furthermore, MSC injected mice retinas showed reduced neovascularization and vaso-obliteration where as

Lin- injected retinas did not result in significant revascularization.
Conclusion: MSCs migrate to the retina in mice having undergone the OIR model and promote proper vascular repair. These results suggest that MSCs play a role in angiogenesis and could have a therapeutic use in retinopathy of prematurity.

140. Endothelial Microparticles Promote Oxidative Stress and Inflammation in Cultured Mouse Aortic Endothelial Cells: Role of Epidermal Growth Factor Receptor

Burger D, Thompson CS, Touyz RM. Kidney Research Centre, Ottawa Hospital Research Institute, University of Ottawa, Canada
Corresponding Author: dburger@uottawa.ca
Conflict of Interest: None declared

Background: Microparticles (MPs), submicron fragments of cellular membranes shed from stressed/damaged cells are found in the plasma of healthy individuals with levels increased in vascular injury. Elevated plasma MP levels correlate with vascular dysfunction and predict future cardiovascular events. However, whether MPs themselves contribute to endothelial dysfunction is unclear.
Objectives: We tested the hypothesis that endothelial MPs, through EGFR, influence endothelial cell (EC) function by increasing EC oxidative stress and stimulating pro-inflammatory responses.
Methods: Endothelial MPs were isolated from the media of cultured mouse aortic ECs by centrifugation and quantified by flow cytometry. ECs were treated with endothelial MPs (10^5/ml) and effects on ROS generation (DHE HPLC), pro-inflammatory signaling (adhesion molecule expression) and inflammatory responses (macrophage adhesion) were examined.
Results: Endothelial MPs significantly increased production of superoxide anion in ECs (~2-fold, $P<0.05$) after 4 hours and expression of the cellular adhesion molecules PECAM (~3-fold, $P<0.05$), and VCAM (~3-fold, $P<0.05$) after 8 hours. Treatment with endothelial MPs (10^5/ml) significantly increased macrophage adhesion to ECs after 8 hours ($P<0.05$). Examination by confocal microscopy suggested a surface interaction between ECs and MPs. Additionally, western blot analysis identified the epidermal growth factor receptor (EGFR) ligand HB-EGF. We therefore tested the hypothesis that MPs promote oxidative stress and inflammation through EGFR activation. Co-treatment with the EGFR inhibitor gefitinib (1 μM), blocked MP-induced oxidative stress and inflammation.
Conclusions: In summary, we demonstrate that endothelial MPs are pro-oxidative and pro-inflammatory in ECs. These effects appear to be mediated through surface interaction and stimulation of EGFR. Thus MPs may be more than just biomarkers of vascular injury and may themselves contribute to endothelial dysfunction.

141. Hair Analysis as a Tool for Estimating Child Exposure to Environmentally Relevant Polybrominated Diphenyl Ethers (PBDEs)

Carnevale A[1,2], Aleksa K[1,3], Koren G[1,2,4]. [1]Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto, Canada; [2]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Canada; [3]School of Pharmacy, University of Waterloo, Waterloo, Canada; [4]Ivey Chair in Molecular Toxicology, University of Western Ontario, London, Canada.
Corresponding Author: jacques.turgeon.chum@ssss.gouv.qc.ca
Conflict of Interest: None declared

Background: Polybrominated diphenyl ethers (PBDEs) are chemicals that are added to a variety of consumer products as flame retardants. They are persistent in the environment and have been detected in wildlife and in humans. The human body burden in North America is among the highest in the world. Some studies have reported higher levels of PBDEs in children, than in their mothers, as measured in serum. PBDEs are structurally similar to other persistent organic pollutants, some of which can potentially interfere with endocrine pathways. This is of concern since childhood is an ongoing period of growth and development.
Objectives: To establish a method to quantify PBDEs in the hair of children (newborn-age 16) as a biomarker of long-term systemic exposure.
Methods: A method has been developed using gas chromatography with mass spectrometry (GC/MS). Fifty mg of children's hair is incubated overnight at 40°C with 4N HCl and hexane (4:1) to extract PBDEs. Samples are eluted from 2g $NaSO_4$: 2g Florosil SPE columns with 8mL of hexane. Samples are then analyzed by GC/MS for PBDE congeners;

BDE-28, -47, -99, -100, -153, -154, -183 and -209.
Results: The total amount of PBDEs varied among samples. Several congeners could be detected in newborn hair. BDE-209 was present in some samples. The greatest variability was seen with congeners BDE-47 and BDE-99.
Conclusions: This method offers a noninvasive technique for assessing chronic exposure to PBDEs in children, and to examine correlations between systemic exposure and adverse developmental outcomes.

142. Impact of Hypertension on Endothelial CaMKII Isoforms from Mesenteric Arteries

Charbel C[1,2], Toussaint F[1], Blanchette A[1], Villeneuve LR[1], Ledoux J[1,3]. [1]Centre de Recherche, Institut de Cardiologie de Montréal, Montréal, QC, Canada, [2]Pharmacologie, [3]Médecine, Université de Montréal, Montréal, QC, Canada.
Corresponding Author: chimenecharbel@gmail.com
Conflit of Interest: None declared

Calcium signaling is fundamental for endothelial functions and an alteration in the regulatory mechanism can lead to cardiovascular pathologies. Recently, spontaneous calcium oscillations localized within the myoendothelial junctions have been identified and named calcium pulsars. Calcium/Calmodulin Kinase II (CaMKII) is a potential target for Ca2+ pulsars involved in endothelial function. Indeed, CaMKII has recently been suggested to play an important role in endothelial function and dysfunction. CaMKII is characterized by its unique ability to interpret intracellular calcium oscillations frequency into specific outcomes. Therefore, alteration in endothelial CaMKII functions might be related with intracellular calcium oscillations such as calcium pulsars. The aim of this study is to investigate the relationship between CaMKII and calcium pulsars and it's alteration in hypertension. Acute and chronic stimulation of calcium pulsars with phenylephrine appears to increase CaMKII activation in endothelium from murine mesenteric arteries. Moreover, CaMKII translocates following calcium pulsars stimulation. Although found in clusters in hypertensive mouse, CaMKII is homogeneously distributed in the endothelium from CTRL mice. qPCR experiments revealed that all four CaMKII isoforms expression (α, β, γ and δ) are significantly diminished (25-57%) in mesenteric arteries from hypertensive mice. In summary, the increase of calcium pulsars frequency in hypertensive mice correlates with CaMKII's distribution in endothelial cells, suggesting their activation by calcium pulsars. Therefore, an altered relationship between CaMKII and calcium pulsars can potentially be involved in endothelial dysfunction associated to hypertension.

143. Development and Pharmacological Characterization of a New Class of Peptidic Urotensin II Antagonists

Chatenet D[1,2], Nguyen QT[3], Létourneau M[1,2], Dupuis J[3,4], Fournier A [1,2]. [1]Laboratoire d'études moléculaires et pharmacologiques des peptides, Université du Québec, INRS – Institut Armand-Frappier, Ville de Laval, Québec, Canada; [2]Laboratoire International Associé Samuel de Champlain (INSERM – INRS); [3]Montreal Heart Institute, Université de Montréal, Montréal, Québec, Canada; [4]Department of Medicine, Université de Montréal, Montréal, Québec, Canada
Corresponding Author: david.chatenet@iaf.inrs.ca
Conflict of Interest: None declared

Background: Known antagonists of the urotensin II (UII) receptor (UT) are of limited utility for investigating the pathophysiological role of UII due to poor potency and limited selectivity.
Objectives: Design, synthesis and pharmacological evaluation of new UII antagonists.
Methods: The pharmacological properties of a novel UT antagonist, [Bip4]URP, was investigated in vitro and in vivo using several bioassays.
Results: Competitive binding assays demonstrated the ability of [Bip[4]]URP to fully displace the radioligands ^{125}I-hUII and ^{125}I-URP from human recombinant UT. In ex vivo studies, various concentrations of [Bip[4]]URP did not produce any significant rightward shift of the hUII concentration-response curve, but the maximal response to hUII was not attainable. Interestingly, a slight but non significant rightward shift, along with a non significant reduction of efficacy, was observed with URP. Supporting these observations, dissociation experiments revealed the propensity of this compound to accelerate the dissociation rate of hUII but not URP, suggesting the presence of two binding pockets in close vicinity. In vivo, [Bip[4]]URP had no effect on the biphasic response induced by URP but significantly reduced the hypotensive activity, while keeping intact the pressor effect induced by hUII.

These results demonstrated its selectivity against hUII agonistic effect.
Conclusion: The results demonstrated the ability of [Bip[4]]URP to reduce the efficacy of hUII- but not URP-induced vasoconstriction. Moreover, in vivo studies support the in vitro pharmacological profile described above, making [Bip[4]]URP as the first peptidic analog of a new class of urotensinergic antagonists.

144. Ethics Involved in Termination of a Wanted Pregnancy in Reproductive Toxicology

Chaudhry S, Koren G, Nulman I. The Motherisk Program, Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto, Canada
Corresponding Author: mueez3@hotmail.com
Conflict of Interest: None declared

Background: A serious conflict is created when a woman must decide whether or not to terminate a wanted pregnancy after exposure to a teratogen. She must weigh the fetal/child risks following exposure against ethical, religious, and psychosocial issues of pregnancy termination.
Objective: To identify the medical, ethical, cultural and spiritual aspects involved in the process of decision-making regarding the termination of a wanted pregnancy after exposure to a teratogen and to provide guidelines for optimal management.
Methods: A case discussion took place including Clinical Pharmacology fellows and staff, graduate and post graduate students, counselors, obstetricians, bioethics faculty, and representatives of all religious Chaplains of the Hospital for Sick Children, in order to discuss and find better ways to support women in their decision making process.
Summary: 1. Woman should be explained her legal rights in decision-making, including the fact that abortion is legal and free in Canada. 2. One-on-one counseling on fetal risk following exposure should be provided based on evidence-based information. 3. The role of the father and family members in supporting the woman's decision making should be stressed. 4. Religious scholars should be involved for spiritual support. 5. In the event of termination, long term support from medical, religious, psychosocial, and family members should be provided.
Conclusion: Collaboration of healthcare providers, bioethics and chaplaincy representatives, and family members, is essential to reduce the women's moral distress when faced with a decision of pregnancy termination after teratogen exposure.

145. Benzodiazepine Use in the Grand-Duchy of Luxembourg from 1995 to 2006: Proposed Definitions of High Dosage Use and Abuse

Cloos J-M[1], Bocquet V[2], Rolland-Portal I[3], Koch P[3], Chouinard G[4]. [1]ZithaKlinik, Luxembourg; [2]CRP-Santé, Luxembourg; [3]Contrôle Médical, Ministry of Social Security, Luxembourg; [4]Department of Psychiatry and Medicine, McGill University Health Center, Montréal, Canada
Corresponding Author: cloosj@pt.lu
Conflict of Interest: None declared

Background: Prevalence rates of benzodiazepine intake vary among studies, mainly due to definitions of benzodiazepine use, abuse, lengths of observation period and samples of subject.
Objective: To examine short- and long-term use of benzodiazepines in a large national sample.
Methods: A 12-year population-based study was conducted in the country of Luxembourg by looking at benzodiazepine prescriptions for all insured subjects in the national health system from 1995 (n =387,862) to 2006 (n =449,972). Patterns of benzodiazepine use and characteristics of benzodiazepine users were studied.
Results and conclusion: The overall number of Defined Daily Dose (DDD) per 1000 insured subjects per day was 82.9; the five most prescribed benzodiazepines were lormetazepam, lorazepam, alprazolam, bromazepam and loprazolam. Alprazolam was the only benzodiazepine showing a threefold increase in its annual prescribed volume during the study period. Subjects having had at least one benzodiazepine prescription (n=236,263) in the 12-year study period, were divided into 3 groups: 1) a "short term delivery" group (34.9%) with benzodiazepine prescription ≤ 3 months; 2) a "discontinuous delivery" group (38.4%); and 3) a "continuous delivery" group (26.7%) of subjects who never stopped taking benzodiazepines once prescribed. High-dose use was defined using a new formula to calculate potential abuse, and a constant number of high dose users was found throughout the study period: 5.3% of all users (0.9% of all subjects) had a yearly benzodiazepine intake higher than the maximum recommended dosage.

146. IGF-1 Effects on Oligodendrocyte Survival and Proliferation are Potentiated through PTEN Inhibition

De Paula M, Almazan G. Department of Pharmacology & Therapeutics, McGill University.
Corresponding Author: marcio.depaula.mdp@gmail.com
Conflict of Interest: None declared

Background: Myelin is a multilayer specialized membrane that wraps nerve fibers and insulates them facilitating the communication between neurons. Myelin destruction causes neurological impairments such as those observed in patients with multiple sclerosis. Oligodendrocytes (OLGs) are cells responsible for producing myelin in the central nervous system. Insulin-like growth factor-1 (IGF-1) is essential for OLG growth, maturation and survival. IGF-1 binds to its receptor to activate the PI3K/Akt/mTOR cascade which mediates protein synthesis, important for OLG progenitors (OLPs) growth. PTEN (phosphatase and tensin homologue deleted on chromosome 10) is the major negative regulator of PI3K/Akt/mTOR pathway.
Objectives: Our aim is to assess whether an inhibitor of PTEN, potassium bisperoxo oxovanadate (Phen), could be used as pharmacological tool to increase or potentiate IGF-1 effects on OLP survival and proliferation.
Methods: Primary cultures of OPC were prepared from the brains of newborn Sprague-Dawley rats. Thymidine incorporation and MTT assay were used as an OPC proliferation and cell viability indexes, respectively. OPC protein extracts were resolved by Western Blotting.
Results: We found that Phen alone increased OLP proliferation and potentiated the effects of IGF-1. In addition, IGF-1-stimulated signaling pathways were further increased by Phen treatment including Akt, S6 ribosomal protein and ERK.
Conclusion: Overall our first results suggest a potential role of IGF-1 on OLG proliferation and survival under PTEN inhibition.

147. Potential Use of Synthetic Kinin B1 Receptor Peptide Agonists as Permeability Enhancers for Improving Drug Delivery to Malignant Brain Tumours

Gobeil F jr[1], Côté J[2], Savard M[1], Bovenzi V[1], Dubuc C[1], Lepage M[2], Fortin D[3]. [1]Dept. of pharmacology; [2]Dept. of nuclear medicine and radiobiology; [3]Dept. of surgery, Univ. de Sherbrooke, Canada, J1H 5N4.
Corresponding Author: Fernand.Junior.Gobeil@USherbrooke.ca
Conflict of Interest: None declared

Background: Some evidence suggests that kinin B1 receptors (B1R), an inducible G protein-coupled receptor (GPCR), can regulate blood-brain barrier (BBB) permeability, including that of brain tumours.
Objectives: 1) To analyze expression of B1R in brain tumour of syngeneic F98 glioma-implanted Fischer rats. 2) To determine whether the SarLys[DPhe^8]desArg^9-bradykinin (NG29), a stabilized kinin B1R agonist, modulates blood brain barrier (BBB) function, thereby improving delivery of macromolecules directly into brain tumours.
Methods: Expression of B1R (mRNA, protein) in brain normal and tumour tissues was determined by RT-PCR, Western blot, and IHC. Effects of NG29 were monitored by non invasive MRI with Gadolinium-based contrast agents Gd-DTPA (0.5 kDa) and Gadomer (17 kDa) (T1-weighted imaging), and by IHC of endogenous albumin (~66 kDa).
Results: Preferential expression of B1R at tumour cells and surrounding tumour vasculature were detected. Intracarotid infusion of NG29, in contrast to natural LysdesArg^9-bradykinin, elevated brain distribution and uptake profiles of intravenous contrast agents within rat glioma and brain surrounding. These effects were dose-dependent, reversible (lasting< 2h), and were blocked by B1R antagonist R892 and non-selective COX inhibitor indomethacin, but not by B2R antagonist HOE140 and NO-synthase inhibitor L-NA. Consistent with MRI data, immunostaining for extravasated albumin at the invasive tumor edge was increased in NG29-treated rats.
Conclusion: Our results document a novel GPCR signalling mechanism for promoting transvascular delivery into brain tumour, involving possibly COX byproducts. They also underline the potential value of synthetic B1R agonists as selective tumour BBB permeabilizers for local delivery of different-sized therapeutics at (peri)tumoral sites.

148. Acyclovir is a Substrate for the Human Breast Cancer Resistance Protein (BCRP)

Gunness P[1,2], Aleksa K[1,3], Koren G[1,2]. [1]Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, M5G 1X8, Canada; [2]Graduate Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College Street, Toronto, Ontario, M5S 3M2, Canada; [3]School of Pharmacy, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, N2L 3G1, Canada. Corresponding Author: patrina.gunness@utoronto.ca
Conflict of Interest: None declared

Background: The renal transport mechanisms of acyclovir have not been fully elucidated. The human breast cancer resistance protein (BCRP) is a transporter that is widely expressed in human tissues, including the kidney. Studies illustrate that, in mice, BCRP mediates the transport of acyclovir into breast milk. It is plausible that acyclovir is a substrate for human BCRP, and hence, the transporter may be actively involved in the tubular efflux of acyclovir. The role of human BCRP in the transport of acyclovir has not been previously evaluated.
Objectives: To determine whether acyclovir is a substrate for the human BCRP.
Methods: Cellular accumulation studies were conducted to determine whether acyclovir is a substrate for the human BCRP. Transfected human embryonic kidney (HEK293) cells [containing the full-length human *ABCG2* gene encoding the wildtype ABCG2 amino acid sequence] were exposed to [8-^{14}C] acyclovir in the presence or absence of the BCRP inhibitor, fumitremorgin C (FTC) for 2 hours. Intracellular [8-^{14}C] acyclovir accumulation was then assessed using a liquid scintillation counter.
Results: The results illustrated that acyclovir is a substrate for human BCRP. In the presence of FTC, there was a 5-fold increase ($p<0.05$) in the intracellular accumulation of acyclovir.
Conclusions: The study is the first to illustrate that acyclovir is a substrate for the human BCRP. The results suggest that BCRP may play a significant role in the renal clearance of acyclovir, and contributes to the further understanding of the tubular transport of acyclovir. Future studies are required to determine the affinity of the transporter for the antiviral agent.

149. The Effect of N-acetylcysteine on the Antitumour Efficacy of Ifosfamide in a Mouse Xenograft Model

Hanly L[1], Chen N[1], Rieder M[1,3,4], Yeger H[6], Koren G[1,2,3,5,7]. [1]Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada; [2]Ivey Chair in Molecular Toxicology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada; [3]Department of Paediatrics, University of Western Ontario, London, Ontario, Canada; [4]CIHR-GSK Chair in Paediatric Clinical Pharmacology, Children's Health Research Institute, London, Ontario, Canada; [5]Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children, Toronto, Ontario, Canada; [6]Divisions of Hematology/Oncology, Hospital for Sick Children, Toronto, Ontario, Canada; [7]Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada.
Corresponding Author: lhanly@uwo.ca
Conflict of Interest: None declared

It is estimated that 82% of children will survive childhood cancer, up from 50% decades ago. While promising, this increased life span has brought awareness of the high potential that these patients may develop late effects, conditions secondary to cancer therapy. Nephrotoxicity is one such late effect, affecting children treated with the chemotherapy drug ifosfamide, commonly used to treat pediatric solid tumours. While effective, in children it is associated with a 30% risk of developing nephrotoxicity, with 5% of this group developing Fanconi's syndrome. N-acetylcysteine, as synthetic thiol that is currently used clinically in children, has successfully mitigated this renal toxicity in both cell and rodent models. However, before this treatment can be realized clinically, we must demonstrate that it does not interfere with the antitumour efficacy of ifosfamide. Our objective is to compare the efficacy of ifosfamide with and without concurrent n-acetylcysteine therapy in an immunocompromised mouse xenograft model. Female Swiss nu/nu mice will be injected with Ewing's Sarcoma tumour cells. They will receive 60mg/kg injections of ifosfamide (3 days), with or without 1.2g/kg injections of n-acetylcysteine (concurrently and for + 3 days). Tumour volumes will be measured. Preliminary data assessing the efficacy of ifosfamide during concurrent n-acetylcysteine therapy *in vitro*, in relevant tumour

cell lines (rhabdomyosarcoma and neuroblastoma) indicate n-acetylcysteine has no effect. We anticipate similar findings with our rodent model (to be completed before May). Upon anticipated results, these findings will demonstrate the clinical applicability of n-acetylcysteine for ifosfamide-induced nephrotoxicity and strengthen support for a clinical trial.

150. The Relationship between Folic Acid Supplementation and Serum Folate Level in Early Pregnancy and Pregnancy Outcomes: MOCEH (Mothers and Children's Environmental Health) Study

Hong S-C[1,2], Ha E-H[3], Han J-Y[4], Park H[3], Ha M[5], Kim Y[6], Hong Y-C[7], Nava-Ocampo AA[1], Koren G[1]. [1]Motherisk Program, Hospital for Sick Children, Toronto, ON, Canada, [2]Korea University Medical Center, Seoul, South Korea , [3]Department of Preventive Medicine, School of Medicine, Ewha Medical Research Center, Ewha Womans University, [4]Korean Motherisk Program, Cheil General Hospital and Women's Healthcare Center, Seoul, South Korea, [5]Department of Preventive Medicine, Dankook University College of Medicin, [6]Department of Occupational and Environmental Medicine, Ulsan University Hospital, University of Ulsan College of Medicine, [7]Department of Preventive Medicine, Seoul National University College of Medicine
Corresponding Author: novak082@korea.ac.kr
Conflict of Interest: None declared

Background: The currently recommended guidelines to minimize the incidence of NTD are well established; serum/plasma folate >7 ng/mL (15.9 nmol/L) or RBC folate concentrations >906nmol/L. However, there is controversy regarding the relationship between serum folate/folic acid supplement use and pregnancy outcome.
Objective: To evaluate the relationship between serum folate/folic acid supplement use and pregnancy outcome.
Methods: As part of the Prospective Cohort Study of the MOCEH, 1220 pregnant women were enrolled. Information about folic acid supplementation, maternal serum folate levels in the 1st & 3rd trimester of pregnancy, and pregnancy outcomes, were obtained. Comparison of pregnancy outcomes between women who received prenatal folic acid supplementation and those who did not receive supplementation was conducted. A post-hoc analysis was performed comparing pregnancy outcome a) between women with blood folate levels <7 ng/mL vs. >7 ng/mL, and b) between women with <20 ng/mL vs. >20 ng/mL folate levels.
Results: The mean and median level of serum folatel were 11.7 ng/mL and 9.6 (95% CI 3.3-26.9), respectively. Overall 32.9% (n= 401/1220) of early pregnant women were at <7ng/mL folate. Only 29.8% (n= 363/1220) of pregnant women took multivitamins with folic acid during the 1st trimester of pregnancy. Serum folate level was higher in the FA supplement group than in the no-FA supplement group. No differences in pregnancy outcomes were observed a) between women who received folic acid supplementation and those who did not, and b) between women with blood folate levels <7 ng/mL vs. >7 ng/mL. Although most preterm deliveries <34 weeks were observed in the group of serum folate <20 ng/mL, there was no statistical significance. Other pregnancy outcomes such as preeclampsia, gestational diabetes, placenta previa and premature rupture of membrane remained similar between groups.
Conclusions: Although most preterm deliveries was observed in the low serum folate levels (<20 ng/mL), there was no significant relation between serum folate level/folic acid supplementation and pregnancy outcomes. This is possibly due to unsufficient sample size, and should be repeated with larger cohorts.

151. NK1 Receptor Antagonists Modulate the Effects of Immunosuppressive Drugs on T Cells Activation: An *in vitro* Study

Jizi K[1], Welman M[1], Maghni K[1]. [1]Centre de recherche de l'hôpital du Sacré-Coeur de Montréal, Université de Montréal, Montréal, Qc, Canada
Corresponding Author: khadije.jizi@umontreal.ca
Conflict of Interest: None declared

Introduction: Cyclosporin A (CsA) and Tacrolimus (FK506) both inhibit T cell activation to prevent or reduce graft reject. Rapamycin also regulate T cells function by repressing cell proliferation. Aside from their therapeutic efficiency, these molecules have several side effects. An option to reduce their adverse effects is to decrease the dose of immunosuppressive drugs by combining it with another molecule. The NK1 receptor (NK1R) expressed on T cells may be a good candidate

because this receptor under activation by its ligand, the substance P (SP), regulates T cells function. The goal of this study is to demonstrate the potential efficacy of combining a NK1R antagonist (L-733,060) with CsA, FK506 or rapamycine.
Methods: Jurkat T cells were incubated with rapamycin (1; 0,5 and 0,1 nM) in presence of a NK1R antagonist L-733,060 (1; 5 and 10 uM). The level of phosphorylation of the S6 ribosomal protein (S6R) was measured by flow cytometry. Jurkat cells were also incubated with CsA (1, 5 and 10 ng/ml) or FK506 (0,01; 0,05 and 0,1 ng/ml) with the L-733,060. The modulation of IL-2 production by T cells was measured by ELISA.
Results: The combination of rapamycin with L-733,060 did not affect the level of phosphorylation of the S6R protein. On the other hand, the combination of CsA or FK506 with L-733,060 significantly reduces IL-2 production in stimulated T cells.
Conclusion: The NK1R signalling pathway is implicated in the production of IL-2 in activated T cells. Combining a NK1R antagonist with immunosuppressive drugs may be an interesting alternative to reduce therapeutic doses without affecting therapy efficacy.

152. Pharmacokinetics of Obesity in Childhood – A Review

Jong GW, Koren G. Division of Clinical Pharmacology & Toxicology, Hospital for Sick Children, Toronto ON
Corresponding Author: Geert.Jong@sickkids.ca
Conflict of Interest: None declared

Introduction: The obesity epidemic is widely recognized as one of the biggest health threats of the modern age. It is not uncommon for clinicians in primary or secondary care to treat illness in an obese child, which will require treatment with a drug for which there is no guide regarding which body size metric to use in determining the optimum dose for this group of patients. We conducted a review of available literature to determine availability of pharmacokinetic data in obese children.
Methods: A review of available adult and paediatric obesity pharmacokinetic literature was conducted.
Results: Definitions of obesity are even more challenging in children than in adults. Many measures of body composition are available, but have not been extensively verified. Whereas absorption seems not to be affected, distribution, metabolism and elimination are known to be in various extend. Plasma protein levels and binding are comparable to non-obese, and effects on regional blood flow are unclear. Evidence suggests that hydrophilic drugs whose V_d in normal-weight subjects is small should be administered according to ideal body weight, and not total body weight. CYP450 enzyme activity is altered for the 2E1 isoform, but for others results are conflicting. Glomerular filtration rates are mostly similar, but this is unclear for tubular reabsorption.
Conclusions: There is a lack of available pharmacokinetic data for the determination of optimal dosing schedules for obese children, and adults alike.

153. Pharmacogenetics of Fatal Paediatric Obstructive Sleep Apnea Cases in Response to Codeine: Additional Cases

Kelly L[1], Rieder M[2], Veenstra F[3], Madadi P[4], Ross C[5], Sistonen J[6], Koren G[7,8]. [1] Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON; [2] Department of Pediatrics and Medicine, Children's Hospital of Western Ontario, University of Western Ontario, London, ON; [3]Grey Bruce Health Services Hospital; [4] Department of Pharmacology, Hospital for Sick Children, University of Toronto, ON; [5] Department of Medical Genetics, Centre for Molecular Medicine and Therapeutics, University of British Columbia; [6]Centre for Molecular Medicine and Therapeutics, University of British Columbia; [7] Motherisk Program, Hospital for Sick Children, Toronto, ON;[8] Ivey Chair in Molecular Toxicology, University of Western Ontario, London, ON
Corresponding Author: lkelly27@uwo.ca
Conflict of Interest: None declared

The primary treatment for pediatric obstructive sleep apnea (OSA) is removal of the tonsils and adenoids (adenotonsillectomy). Codeine is prescribed for the pain associated with this procedure. Codeine is a prodrug which requires conversion to morphine via the highly polymorphic cytochrome P450 (CYP) 2D6 enzyme to elicit its analgesic properties. A functional gene duplication causes increased morphine production in the ultra-rapid metaboliser (UM) phenotype. In 2009 we reported a case of fatal respiratory depression in a 2yr old male post-adenotonsillectomy with toxic morphine levels.

Post-mortem analysis revealed bronchopneumonia and a CYP 2D6 UM phenotype. We report now 2 additional Ontario cases. In September 2010 we recorded a near fatal case of respiratory depression in a 3yr old female OSA patient. She arrived in hospital comatose with toxic blood morphine levels despite using recommended codeine doses. Upon mechanical ventilation and standard naloxone treatment she fully recovered. Salivary genetic analysis revealed that she was a CYP 2D6 UM. A third, fatal case was encountered in November 2010, again, with toxic blood morphine levels after taking only recommended doses. This case of respiratory depression occurred in a 4 yr old male. This patient had a previous history of asthma and was identified as a CYP 2D6 UM. We hypothesize that the combination of an UM CYP 2D6 phenotype and an existing respiratory condition in toddlers with OSA increases the risk for central nervous system depression upon standard codeine administration.

154. MTHFR C677T Polymorphism and the Risk of Colorectal Cancer: A Systematic Review and Meta-Analysis

Kennedy DA[1,2], Stern SJ[1,3], Sarkar M[1], Adams-Webber T[1], Koren G[1,2,3]. [1]The Motherisk Program, The Hospital for Sick Children, Toronto, ON, Canada; [2]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada; [3]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada
Corresponding Author: dkennedy@ccnm.edu
Conflict of Interest: None declared

Background: Folates are water soluble B vitamins that are cofactors in thymidine and purine synthesis and DNA methylation pathways. Methylenetetrahydrofolate reductase (MTHFR) is key in the remethylation of homocysteine to methionine. In 1995 a variant of the MTHFR enzyme, genotype TT, was identified with reduced activity versus CC, causing an accumulation of homocysteine and higher rates of thymidine synthesis. Individuals with this variant are thought to be at a reduced risk for colorectal cancer.
Objective: This meta-analysis examines whether a relationship exists between the variants of MTHFR C677T gene, folic acid intake and the risk of colorectal cancer.
Methods: A systematic review and meta-analysis were conducted. MEDLINE, Embase and SCOPUS were searched from inception to May 2010 with the following search terms "folic acid", "C677T", methylenetetrahydrofolate reductase", "colorectal cancer", "colonic neoplasms", "rectal neoplasms". Observational studies in adult populations were included that reported MTHFR C677T variants, defined levels of folic acid and the risk of colorectal cancer.
Results: Out of 633 records, 28 studies met our inclusion criteria. Preliminary results indicate that the summary risk estimate for case control studies comparing MTHFR CC to CT was 0.92 (CI 95% 0.83-1.01) with some heterogeneity. The summary risk estimate for case control studies comparing MTHFR CC to TT was 0.82 (CI 95% 0.71-0.95) with some heterogeneity.
Conclusion: This meta-analysis supports the association between the TT genotype and a reduced risk of colorectal cancer. Further analysis is required to provide insight into whether this risk is further modified through folic acid intake levels.

155. Transcriptional Regulation and Function Organic Anion Transporting Polypeptide 2B1 Splice Variants

Knauer MJ[1], Girdwood AJ[1], Kim RB[1,2], Tirona RG[1,2]. [1]Department of Physiology and Pharmacology; [2]Department of Medicine, Division of Clinical Pharmacology, Lawson Health Research Institute, The University of Western Ontario, London, Ontario, Canada. Corresponding Author: rommel.tirona@schulich.uwo.ca
Conflict of Interest: None

Background: The human Organic Anion Transporting Polypeptide 2B1 (OATP2B1) is a membrane transporter that facilitates the cellular uptake of a number of endogenous compounds and drugs. OATP2B1 is expressed in several tissues including the small intestine, liver, kidney and skeletal muscle. Recently, it has been shown that differential promoter usage in tissues results in the expression of several OATP2B1 splice variants which utilize 5 distinct first exons but share common subsequent exons. These splice variations are expected to encode either a full length or truncated protein missing 22 amino acids from the N-terminus. Since little is known about OATP2B1 splice variants we investigated the relative expression of the splice variants in key tissues responsible for drug absorption and elimination, as well as the transport

function of the truncated variant.
Methods and Results: Using variant-specific polymerase chain reaction, both the predicted full length and truncated forms of OATP2B1 were detected in liver, kidney and small intestine, albeit in differing proportions. With heterologous expression in cultured cells, we compared the transport kinetics (Vmax and Km) of the two forms of OATP2B1. Using cell based reporter assays we determined that HNF4α was able to transactivate transcription of the truncated variant but not the full length form. Importantly, we demonstrate that the truncated variant was capable of transporting the known OATP2B1 substrates, estrone sulfate and rosuvastatin.
Conclusion: These findings indicate that differential regulation of OATP2B1 splice variant expression in tissues could contribute to variation in drug response.

156. Digoxin Toxicity Precipitated by Clarithromycin Use: Case Presentation and Review of the Literature

Lee CYW,[1] Marcotte F,[1] Crépeau J,[1] Giraldeau G,[1] Tardif J-C,[1] Koren G,[2] Juneau M[1] [1]Montreal Heart Institute, Université de Montréal, Montreal, Quebec, [2]University of Toronto, Toronto, Ontario
Corresponding Author: Candace.Lee@icm-mhi.org
Conflict of Interest: None declared

An 83-year-old man with a past history of hypertension, dyslipidemia, myocardial infarction, and left ventricular dysfunction presented with a recent history of fatigue, dyspnea on exertion, cough, nausea, loss of appetite, and lightheadedness. Three days prior to his presentation, the patient had been started on clarithromycin 500 mg p.o. b.i.d. for treatment of possible pneumonia. His cardiac medications included digoxin 0.125 mg p.o. q.d., spironolactone 25 mg p.o. q.d., captopril 25 mg p.o. b.i.d., pravastatin 40 mg p.o. q.d., isosorbide mononitrate 30 mg p.o. q.d., furosemide 60 mg p.o. q.d., bisoprolol 2.5 mg p.o. q.d., and potassium 20 mmol p.o. q.d. Due to severe nausea, the patient presented to the Emergency Department following completion of his fifth dose of clarithromycin. On examination, he appeared unwell. Vital signs were stable. Cardiorespiratory examination did not show signs of decompensated heart failure. Blood tests demonstrated a white cell count of 13.6 x 10^9/L, potassium 5.2 mmol/L, creatinine 184 µmol/L (creatinine was normal 3 weeks before), and digoxin levels of 4.6 nmol/L (5 hours post-dose) and 4.7 nmol/L (18 hours post-dose). Ventricular extrasystoles were observed during monitoring. The presentation of this patient was consistent with digoxin toxicity in the context of renal dysfunction and concomitant use of the macrolide antibiotic, clarithromycin, which is known to inhibit P-glycoprotein-mediated efflux mechanisms of digoxin. Both drugs were discontinued. The patient was hospitalized and was discharged ten days later. Health care providers need to be vigilant of this potential drug-drug interaction. A focused literature review will be presented.

157. Drug Use Reviews (DUR) in Non-Institutional Settings

Levine MAH, Syed U. Centre for Evaluation of Medicines, St. Joseph's Healthcare and Dept. of Clinical Epidemiology & Biostatistics, McMaster University
Corresponding Author: levinem@mcmaster.ca
Conflict of Interest: None declared

Background/Objective: Drug use review (DUR) has become an increasingly popular component in budget activities conducted by drug benefit programs that operate in the non institutional environment. There is great diversity in the methods used to conduct drug use reviews, and each method has different strengths and weaknesses. This review was designed to determine the circumstances under which these methods are used.
Methods: A literature search was conducted in MEDLINE, EMBASE and CINAHL databases to identify original studies that assessed prescription drug use in a community, published between January 2007 and February 2010. Each article was assessed for (a) data source i.e., patient, pharmacy or prescriber; (b) drug issue of interest; (c) method used for data accrual.
Results: 587 articles involving 632 DUR strategies fulfilled the criteria for inclusion in the analysis. 81% were retrospective studies. The source of the prescription drug use information was obtained from pharmacy or dispensing claims data 52%, the patient 27% and physician records 21%. The most common goal was the assessment of drug use patterns by population demographics (n=151). Measuring adherence and misuse of prescription by patients was second (n=136) and physician adherence to

prescription guidelines or recommendations was third (n=120). When data was collected at the patient level the elapsed time between drug use and the survey was more than one month 33% of the time, and in 40% of studies the time period was not specified; drug use information was reported by a proxy 11% of the time.
Conclusion: Most DUR studies were conducted retrospectively using prescription dispensing claims data to address simple quantitative questions. Studies designed to address questions pertaining to the quality of prescribing often used patient reported data to obtain information that would not be available from administrative databases but these were conducted using weaker methodologies.

158. Investigating the Genetic Causes Associated with Incristine-induced Neurotoxicity in Children

Loo TT[1-3], Ross CJD[4,5], Rassekh SR[6], Hayden MR[4,5], Carleton BC[1-3]. University of British Columbia Faculty of Medicine, Department of Paediatrics[1]; Pharmaceutical Outcomes Programme, BC Children's Hospital[2]; Child & Family Research Institute, Vancouver[3]; UBC Centre for Molecular Medicine and Therapeutics[4]; UBC Faculty of Medicine, Department of Medical Genetics[5]; Paediatric Hematology/Oncology/BMT, BC Children's Hospital[6]
Corresponding Author: tloo@popi.ubc.ca
Conflict of Interest: None declared

Background: Vincristine is a highly effective oncology drug that is considered to be the standard treatment against many types of cancers. However, the clinical utility of vincristine is significantly limited by debilitating adverse drug reactions (ADRs), such as neurotoxicity, and more specifically, peripheral neuropathy.
Objectives: To identify novel predictive genomic markers and/or clinical factors that will determine an individual's susceptibility to vincristine-induced neurotoxicity.
Methods: DNA samples and detailed clinical data were collected through the Canadian Pharmacogenomics Network for Drug Safety (CPNDS), an active ADR surveillance network encompassing 13 Canadian paediatric hospitals. ADRs were characterized according to a modified version of the Common Terminology Criteria for Adverse Events (v4.03), and stratified by severity, location, and type of neuropathy. Patient samples were genotyped for single nucleotide polymorphisms (SNPs) associated with drug absorption, distribution, metabolism, and excretion. A separate panel containing functional and tagging SNPs of genes that are specifically involved in vincristine's mechanism of action was also developed.
Results: Within CPNDS, 26.3% of paediatric cancer patients on vincristine therapy suffered from vincristine-induced neurotoxicity (305 cases and 854 controls). The neurotoxicity cases have been preliminarily divided into categories of peripheral, autonomic, and central neuropathy.
Conclusions: This study aims to establish the causality of vincristine-induced neurotoxicity in order to assess the risk-benefit ratio of utilizing vincristine for each patient. By identifying individuals who would derive a greater benefit from alternative anti-cancer treatments, vincristine-induced neurotoxicity could potentially be avoided. Additionally, the modified neurotoxicity grading scale and accurate symptom classifications serves to facilitate improvements in the monitoring of vincristine-induced neurotoxicity.

159. Development of an In Vitro System for the Functional Study of Oatp1b1 Transporter of Statins

Lu J, [1,2] Armstrong C,[1,2] Belanger F,[2] Gaudette F, [2] Turgeon J[1,2]. [1]Faculty of Pharmacy, Université de Montréal, Montreal, QC, Canada; [2]CRCHUM, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada.
*Corresponding author***:**
jacques.turgeon.chum@ssss.gouv.qc.ca
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Background and Objectives: OATP1B1 is a known transporter of statins such as rosuvastatin. As statins are widely used to treat high cholesterol, it is important to study their interactions at the cellular level. A mutation in this membrane transporter or drug-drug interactions could modify the plasma and intracellular concentrations of statins which can lead to numerous side effects such as cellular toxicity. The goal of this study was to evaluate the uptake of rosuvastatin by this transporter using a cell line which overexpresses OATP1B1.

Methods: OATP1B1 was cloned into the pIRES-EGFP vector. Site-directed mutagenesis was used to generate the loss-of-function V174A mutant, which was confirmed by sequencing. Stable cell lines were created in HEK293 cells, and selection was done by G418 treatment, followed by selection by Fluorescence Activated Cell Sorting (FACS). OATP1B1 and OATP1B1-V174A expressing cells were then plated on Poly-Lysine treated 6-well plates, and then incubated in the presence of increasing concentrations of rosuvastatin for various time points.
Results: The uptake of rosuvastatin was observed in the presence of the wild type OATP1B1 in as little as 5 minutes. However, the uptake of rosuvastatin in OATP1B1-V174A was not observed, confirming that this mutant is non-functional. Controls were performed to ensure that the background signal of rosuvastatin was minimal.
Conclusions: Stable HEK293 cell lines overexpressing OATP1B1 and OATP1B1-V174A can be used as in vitro models for the functional studies of this transporter. Further studies will involve testing inhibitors of rosuvastatin uptake by OATP1B1.
Keywords: Drug-Drug Interactions, OATP transporters, statin

160. Stereoisomers of Naringenin as Pleiotropic, Selective Inhibitors of Cytochrome P450 Isoforms

Lu WJ[1], Flockhart DA[1], Caccamese S[2]. [1]Department of Pharmacology and Toxicology, Indiana Institute for Personalized Medicine, Indiana University School of Medicine, USA, [2]Dipartimento di Scienze Chimiche, Università di Catania, Italy
Corresponding Author: dflockha@iupui.edu
Conflict of Interest: None declared

Naringenin is the predominant flavonone in grapefruit, and has been suggested to be a bioactive antioxidant, free radical scavenger and potential adjunct to treatment of Hepatitis C in humans. Interactions between naringenin and the cytochrome P450 system have been of interest since the first demonstration that grapefruit juice reduced cytochrome P450 3A activity. The effects of naringenin on other cytochrome P450 isoforms have been less carefully investigated. In addition, the stereoisomers of naringenin have not been separated and purified before, and so the stereoselectivity of naringenin's effects has not been characterized. We isolated pure naringenin enantiomers and used them to test the ability of S-, R-, and racemic naringenin to inhibit a series of key drug metabolizing cytochrome P450 isoforms *in vitro*. We determined the IC_{50} values for each naringenin preparation using *in vitro* incubations with recombinant human cytochrome P450 isoforms. We also tested the ability and stereoselectivity of naringenin to inhibit cytochrome P450-mediated drug metabolism in human liver microsomes. Naringenin was able to inhibit CYP2C9, CYP2C19 and CYP19 with IC_{50} values below 5μM. No substantial inhibition of metabolism by CYP2B6 or CYP2D6 was observed at concentrations up to 10μM. The S-enantiomer exhibited higher inhibitory potency than the R-enantiomer for CYP2C19 and CYP19, while the R-enantiomer was more potent as an inhibitor of CYP2C9. Chiral flavonones like naringenin are difficult to separate into their enantiomeric forms, but stereoselective effects may be observed that impact clinical activity. Inhibition of specific drug metabolizing enzymes by naringenin observed *in vitro* may be exploited to understand pharmacokinetic changes seen *in vivo*.

161. Objective Pharmacological Properties that may Turn a Vasoactive Hormone into an Animal Toxin: Differences between Bradykinin and Maximakinin

Marceau F[1], Bawolak M-T[1], Gera L[2]. [1]Centre de recherche en rhumatologie et immunologie, Centre Hospitalier Universitaire de Québec and Department of Microbiology-Infectious Disease and Immunology, Université Laval, Québec QC, Canada G1V 4G2; [2]Department of Biochemistry, University of Colorado Health Sciences Center, Denver, Colorado 80262, U.S.A. Corresponding Author: francois.marceau@crchul.ulaval.ca
Conflict of Interest: The authors declare no conflict of interest.

Background: Maximakinin, a natural 19-residue peptide from the skin of the amphibian Bombina maxima (DLPKINRKGPRPPGFSPFR), incorporates the full sequence of bradykinin (BK) at its C-terminus with a hydrophilic N-terminal extension. As a putative venom component, it may stimulate BK B_2 receptors (B_2Rs) in a distinct manner relative to the fragile mammalian agonist BK.

Methods: radioligand binding assays ([^{3}H]BK to B_2R-green fluorescent protein (GFP) conjugate, [^{3}H]enalaprilat to angiotensin converting enzyme (ACE)) were applied in HEK 293(a) cells to compare the affinity of maximakinin to that of BK at characteristic molecular targets. Human umbilical vein contractility and imaging/downregulation/ signalling studies of B_2R-GFP in HEK 293 cells were other applied assays of the effects of the peptides at natural or recombinant B_2Rs.
Results: Maximakinin is an agonist of the BK B_2R with a 7-20 fold lesser potency, but a prolonged (≥ 12 h) duration of action relative to BK (ERK MAP kinase activation, c-Fos induction in HEK 293 cells). Maximakinin displaced [^{3}H]enalaprilat binding from recombinant ACE much less effectively than BK. Unlike BK, maximakinin induced the internalization of the fusion protein B_2R-GFP and the downregulation of this construction over a 12-h stimulation period, fully reproducing the effect of synthetic inactivation-resistant B_2R agonists.
Conclusions: Maximakinin has little affinity for ACE, a major ectopeptidase that inactivates BK, and is further resistant to other proteases present in the endosome. It is a natural kinin sequence that elicits a prolonged signalling, a possible basis for a venomous action in nature and for drug development in the laboratory.

162. Ethyl Glucuronide as a Biomarker of Alcohol Consumption During Pregnancy

Matlow, J[1,2], Aleksa K[1,3], Koren G[1,2]. [1]Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto, Ontario, Canada; [2]Graduate Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada; [3]School of Pharmacy, University of Waterloo, Waterloo, Ontario, Canada
Corresponding Author: jeremy.matlow@utoronto.ca
Conflict of Interest: None declared

Background: Alcohol consumption during pregnancy can lead to Fetal Alcohol Spectrum Disorder (FASD), which manifests itself in the form of physiological defects, mental retardation and neurobehavioural deficits, the latter of which is rarely diagnosed. Since maternal self-reports are often unreliable, a biomarker of alcohol use during pregnancy is needed to accurately determine fetal exposure and risk for FASD. While Fatty Acid Ethyl Esters (FAEEs) are current biomarkers of exposure, the introduction of a second biomarker that can be tested alongside FAEEs promises to greatly increase the sensitivity and specificity of analytical screens. Ethyl glucuronide (EtG) is a direct metabolite of ethanol that has been detected in the meconium, or first stool of life, of infants born to mothers who consumed alcohol during pregnancy. Whether this detected EtG was formed by the maternal liver and crossed the placenta, by the fetal liver after ethanol crossed the placenta, or by the placenta itself remains unknown.
Objectives: To determine if, and to what extent EtG crosses the human placenta, and to measure placental formation and degradation of EtG.
Methods: Placentae from consenting women undergoing elective Caesarian section at St. Michael's Hospital in Toronto, Ontario will be taken to the on-site perfusion laboratory. One μg/mL EtG will be added to the maternal reservoir and maternal and fetal samples will be taken over a 3 hour period to determine if and to what degree EtG is crossing the human placenta. EtG will be quantified using HS-SPME GC-MS. Placental metabolism of ethanol to EtG and EtG to ethanol will be investigated using placental microsomes. Kinetic parameters (Vmax, Km) will also be determined to help elucidate the role of the placenta in formation and degradation of EtG.
Results: To date the perfusions are being carried out and the level of EtG in the perfusate will be determined using HS-SPME GC-MS with a limit of sensitivity of 1 ng/vial.
Conclusions: We have developed a HS-SPME method for detecting EtG in maternal and fetal perfusate samples. This will allow us to accurately quantify the transfer of EtG across the human placenta.

163. Cocaethylene as a Biomarker of Alcohol and Cocaine Co-consumption in Human Hair

Natekar A[1,2]; Aleksa K[1,3]; Koren G[1,2,4,5]. [1]Division of Clinical Pharmacology & Toxicology, Hospital for Sick Children, Toronto, ON; [2]Department of Pharmacology & Toxicology, University of Toronto, Toronto, ON; [3]School of Pharmacy, University of Waterloo, Waterloo, ON; [4]Department of Physiology & Pharmacology, University of Western Ontario, London, ON; [5]Ivey Chair in Molecular Toxicology, University of Western Ontario, London, ON

Corresponding Author: aniket.natekar@utoronto.ca
Conflict of Interest: None declared

Background: Cocaethlyene (CE) is a metabolite of cocaine formed only during cocaine and alcohol co-consumption. It is pharmacologically active, prolonging cocaine-related effects.
Objective: To determine if CE can be used as a biomarker in hair testing to indicate alcohol and cocaine co-consumption.
Methods: We used liquid-liquid extraction and solid-phase extraction to isolate cocaine and its metabolites from hair, as well as fatty acid ethyl esters, a direct biomarker of alcohol consumption. The compounds concentrations were analyzed and determined using GC-MS.
Results: Out of 516 individuals who tested positive for cocaine, 336 individuals were confirmed cocaine users. Of these, CE was detected in 73 individuals. From those, only 23 had alcohol testing requested, and 15 tested positive for chronic alcohol abuse. The fraction of cocaine that was converted to CE ranged from 84% at low cocaine concentrations, to about 4% at the higher cocaine concentrations. In a few cases, there were difficulties in identifying CE when compared to benzoylecgonine, another cocaine metabolite, as the two metabolites have similar retention times on GC-MS.
Conclusions: At this stage of our research, about 22% of confirmed cocaine users were positive for CE, indicating alcohol co-consumption. However, other studies have found that, on average, about 62% of confirmed cocaine users also consume alcohol. It is possible that hair accumulation is dose dependent and does not identify low alcohol use.

164. Child Neurodevelopment Following In-Utero Exposure to Maternal Azathioprine: Preliminary Results

Nulman I, Barrera M. The Hospital for Sick Children, University of Toronto
Corresponding Author: irena.nulman@sickkids.ca
Conflict of Interest: None declared

Background: Azathioprine (AZA) is an immunosuppressant drug commonly used by pregnant women. Its effects on the fetal central nervous system remain unknown. The **objective** was to assess child neurocognitive development following in-utero exposure to AZA for maternal Inflammatory Bowel Disease (IBD), and to compare the results to those of three comparison groups.
Methods: Mother/child pairs: Group 1-exposed to AZA, Group 2-exposed to corticosteroids; Group 3-exposed to other IBD medications, Group 4-exposed to non-teratogens. Matching criteria: gender and age at testing (children); age (mothers). Using standardized psychological tests, mother/child pairs were assessed on Full Scale, Verbal and Performance IQs.
Results: No significant differences were found among the 4 groups of children (3-12 years) in Full Scale IQ (Group 1=109.2±8.1, Group 2=108.4±12.0, Group 3=109.9±12.9, Group 4=113.9±13.7), Verbal and Performance IQs. Women on corticosteroids experienced more bleeding episodes during gestation (50%vs3.7%vs22.9% in AZA group and other medications group, respectively). Children exposed to corticosteroids had significantly shorter gestational ages, lower birth weights and spent more days in NICU. The only significant predictor of these outcomes was corticosteroid group affiliation. Later child health parameters (growth, number of infections and other health problems) did not differ among the groups
Conclusions: There were no associations found between AZA exposure, maternal disease complications during pregnancy, and children's cognitive performance, which is reassuring. However, no statistical differences in IQ may be a power issue. The adverse outcomes related to corticosteroids can be explained by maternal disease activity. The study remains in progress.

165. Pharmacokinetic Profiles for Oral and Subcutaneous Methotrexate in Patients with Crohn's Disease

Patel V[1,2], Chande N[1], Ponich T[1], Schwarz UI[2], Dresser GK[2], Tirona RG[2], Kim RB[2], Gregor JC[1]. [1]Division of Gastroenterology, Department of Medicine; [2]Division of Clinical Pharmacology, Department of Medicine; University of Western Ontario, London, Ontario
Corresponding Author: vpatel33@uwo.ca
Conflict of Interest: None declared

Background: Methotrexate is a very effective treatment for both the induction and maintenance of remission in Crohn's disease. It is administered subcutaneously due to concerns about drug absorption in this patient population. Furthermore, significant inter-individual variability in its oral

bioavailability has been demonstrated in patients with rheumatoid arthritis.
Objectives: To compare the pharmacokinetics of oral and subcutaneous methotrexate in patients with Crohn's disease.
Methods: A total of three patients with stable Crohn's disease have been enrolled thus far. Each patient received an upper endoscopy to assess for evidence of upper gastrointestinal Crohn's disease, followed by two pharmacokinetic (pk) studies – oral (PO) methotrexate and subcutaneous (SC) methotrexate. During each study day, the patients received either PO or SC methotrexate followed by plasma collection at 11 pre-specified time points over a 24 hour period. Methotrexate plasma drug concentrations were then obtained using a sensitive mass spectrophotometer.
Results: Three patients have been enrolled in this ongoing study. All three had normal upper endoscopies with normal duodenal biopsies. The mean half-life was 2.8 hours and 3.2 hours for PO and SC methotrexate, respectively. The mean time to maximum plasma concentration was 1.2 hours and 0.5 hours PO and SC methotrexate, respectively. The area under the curve ratio (PO/SC) was 0.821. There were no adverse events.
Conclusions: This unique study demonstrates that the pharmacokinetic parameters for PO and SC methotrexate were comparable in three patients with Crohn's disease. Oral methotrexate would be a more convenient, safer, and less expensive option for these patients.

166. Cardiac Effects of Hyperthyroidism Prevented by Concomitant Amiodarone Administration during Mistaken Thyroid Supplementation

Pollak PT. University of Calgary, Calgary, AB, Canada. *Corresponding Author:* pollak@dal.ca
Conflict of Interest: None declared

Background: Hypo-hyper-thyroid states occur more often in pts taking amiodarone. Its bi-iodinated structure increases iodine stores in pts and inhibits conversion of T4 to T3. It takes >1 year for this iodine to leave the body after stopping drug.
Objectives: To illustrate that not stopping amiodarone when thyroid abnormalities are diagnosed can have beneficial effects.
Methods: A 53 y man treated for atrial fibillation (AF) was monitored regularly (EKG, thyroid, liver, and serum drug monitoring). At 30 mo of therapy, he remained asymptomatic in sinus rhythm, but with elevated freeT4 (42 pmol/L - ULN 25 pmol/L). He was prescribed methimazole 5 mg TID and continued amiodarone.
Results: By mistake, liothyronine 5 μg TID was dispensed without detection for 57 d. Despite this, the pt remained asymptomatic with HR <70 for 53 d before having breakthrough AF when freeT4 reached 93 (3.7 x ULN). Total T3 showed a comparatively minor increase to 3.1 (1.1 x ULN of 2.8 nmol/L).
Conclusion: Although this pt went without prescribed antithyroid treatment, he suffered no hyperthyroid symptoms for 53 d. It appears that amiodarone mitigated the effects of rising T4 by preventing a parallel rise in T3 and maintaining sinus rhythm <70. After 3 mo of methimazole, freeT4 normalized and remains at 20. Hyperthryoidism with amiodarone needs careful monitoring and treatment, but is usually limited to 2-8 mo duration. Stopping amiodarone does not lead to rapid depletion of iodine stores. However, continuing amiodarone at effective doses may prevent many of the cardiovascular consequences of hyperthyroidism.

167. Serum Antibody Titres Against Influenza Virus in Pediatric Patients after Treating with Peramivir

Tanaka T [1,2], Nagai Y[1]. [1]Department of Pediatrics, Hitachi, Ltd., Hitachinaka General Hospital; [2]Department of Pediatrics, Shizuoka Kosei Hospital
Corresponding Author: toshihiro.tanaka@alumni.utoronto.ca
Conflict of Interest: None declared

Background: Influenza virus infection is known to be basically self-limiting; however, severe condition, such as respiratory failure and encephalopathy, can sometimes occur especially in high-risk group of patients, including children, and thorough treatments are required. Peramivir (Rapiacta®, Shionogi & Co., Ltd. / BioCryst), a new neuraminidase inhibitor, administering intravenously, was approved for adults and a clinical trial for children was conducted in Japan during the 2009-2010 influenza season. The data from the trials showed that peramivir is effective and safe enough to use in both adults and children with influenza virus infection.
Objectives: Because it is highly effective and the

time to recover from the infection is shortened after the administration of mostly one dose, it was uncertain whether a patient obtains serum antibody against influenza virus after treating with peramivir.
Materials and Methods: A multicenter and open-label clinical trial without a control group was conducted in children with influenza virus infection during the 2009 pandemic A (H1N1) influenza epidemic in Japan to evaluate the efficacy and safety of peramivir. Peramivir was given intravenously at a dosage of 10 mg/kg (600 mg maximum) once daily. Nine patients (4 m/o - 15.5 y/o, median: 1.2 y/o, male/female: 3/6) were enrolled in our facility and were treated with peramivir within 48 hours (5.6 - 28.6 hours, median: 16.1 hours) after developing fever. Eight patients had single dose and one (4 m/o) had second dose due to persistent fever. Blood samples were provided before and after the administration and serum was separated from each sample and analyzed for the titre of antibody against the 2009 pandemic A (H1N1) influenza virus strain in the hemagglutination inhibition (HI) test.
Results: Ten samples from seven patients were obtained before and next day of the administration (within 60 hours after the onset), and all of them showed negative HI titres less than 1: 10. Four samples from four patients were provided 5 - 7 days after the onset; two showed HI titres 1: 10 and the other two showed less than 1: 10. Nine samples from each patient 1 - 2 months after onset were investigated; all showed positive HI titres (1: 40 – 1: 640, median: 1: 160).
Conclusions: Although peramivir produces rapid recovery from influenza virus infection, it does not seem to interfere with obtaining antibodies in blood.

168. Personalizing Tamoxifen Therapy for Breast Cancer Patients

Teft WA[1], Dingle B[2], Younus J[2], Vandenberg T[2], Brackstone M[2,3], Kim RB [1,2,4]. [1]Department of Medicine, Division of Clinical Pharmacology, [2]Department of Oncology; [3]Department of Surgery, [4]Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada. Corresponding Author: wteft@uwo.ca
Conflict of Interest: None declared

Background: Tamoxifen, a standard endocrine therapy for estrogen receptor positive breast cancer, requires hepatic metabolism by cytochrome P450 2D6 (CYP2D6) to be converted to its active metabolite, endoxifen. Genetic variability and drug interactions can cause alterations in CYP2D6 activity. Studies indicate that plasma levels of endoxifen are significantly lower in CYP2D6 poor metabolizers (PMs) and that PMs have worse outcomes including recurrence rates and survival compared to EMs. However, recently there has been conflicting evidence regarding the usefulness of CYP2D6 genotyping for tamoxifen therapy.
Objectives and Methods: We hypothesize that a patient's CYP2D6 genotype and plasma endoxifen level together will better predict their success on tamoxifen. We are collaborating with the London Regional Cancer Program to refer patients on tamoxifen to provide a blood sample for CYP2D6 genotyping by TaqMan assays and drug level analysis of tamoxifen and endoxifen by LC-MS/MS.
Results: We observe that PMs have the highest tamoxifen plasma concentration compared to intermediate (IMs) and extensive metabolizers (EMs). Endoxifen levels for IMs and PMs are significantly lower than EMs. We observe a 12-fold variability in endoxifen levels among EM patients, suggesting that other factors in addition to CYP2D6 contribute to endoxifen concentration. We are examining the effects of interacting medications by drug level monitoring pre- and post-drug change and dose escalation of tamoxifen in patients within the lowest quartile of endoxifen levels.
Conclusions: Low endoxifen levels irrespective of CYP2D6 genotype may indicate a lack of benefit of tamoxifen therapy and may be a better predictor of therapeutic outcome.

169. Pharmacogenetics of Post Partum Management - A Single Center Experience

vanderVaart S[1,2], Berger H[3], Koren G[1,2,4]. 1Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada; 2Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto, Ontario, Canada; 3Department of Obstetrics and Gynecology, St. Michael's Hospital, Toronto, Ontario, Canada; 4Ivey Chair in Molecular Toxicology, Department of Medicine, University of Western Ontario
Corresponding Author: svandervaart@yahoo.com
Conflict of Interest: None declared

Background: Codeine, a common opioid prescribed for pain post c-section, is biotransformed by the highly polymorphic Cytochrome P450 enzyme 2D6

(CYP2D6). Ultrarapid metabolizers (UM), individuals with multiple active copies of CYP2D6, can biotranform up to 50% more codeine into morphine than normal, resulting in adverse reactions to codeine. In contrast, poor metabolizers (PM), individuals who have no active CYP2D6 genes, convert almost no codeine into morphine and as a result may take multiple doses of codeine without attaining analgesia. It would be optimal if we could titrate a mother's codeine dose depending on her level of pain and CYP2D6 genotype.

Objective: To model the pharmacodynamic effect of codeine on pain levels in CYP2D6 retrospectively-genotyped women recovering from c-section.

Methods: Forty-five codeine-prescribed mothers provided a blood sample for CYP2D6 genotyping and recorded their pain level 4x/day for 3 days immediately following a c-section. Genotyping was completed once mothers had been discharged from hospital. Codeine doses and times were recorded, retrospectively adjusted and modeled using the CYP2D6 genotype activity score developed by Gaedigk et al and the pharmacokinetic data from Kirchheiner et al.

Results: No correlation (n=45, spearman's rho= 0.034, p=0.827) was found between Area under the VAS-time Curve (AUC) for pain and genotype-adjusted codeine dose for the group. However, women at the genetic extremes reported codeine effects consistent with previous literature. The 2 PMs of codeine reported no analgesia as a result of taking codeine, while two of the three UMs reported immediate pain relief from codeine, but stopped taking it due to adverse affects (i.e. nausea, lightheadedness and constipation).

Conclusion: A model to predict the analgesia affect of codeine is more complex than its CYP2D6 genotype score alone.

170. Pharmacogenomic Prediction of Anthracycline-induced Cardiotoxicity in Children

Visscher H[1], Ross CJD[1], Rassekh SR[2], Sistonen J[1], Amstutz U[7], Barhdadi A[3], Dubé M-P[3], al-Saloos H[4], Sandor GS[4], Brown AMK[3,5], Rogers PC[2], Phillips MS[3,5], Rieder MJ[6], Carleton BC[7], Hayden MR[1] & the CPNDS consortium. [1]Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, Department of Medical Genetics, University of British Columbia, Vancouver, Canada; [2]Division of Pediatric Hematology/Oncology/BMT, Department of Pediatrics, University of British Columbia, Vancouver, Canada; [3]Montréal Heart Institute Research Centre and Université de Montréal, Montréal, Canada; [4]Division of Pediatric Cardiology, Department of Pediatrics, University of British Columbia, Vancouver, Canada; [5]Montréal Heart Institute and Genome Québec Pharmacogenomics Centre, Montréal, Canada; [6]Department of Paediatrics, Children's Hospital at London Health Sciences Centre, London, Canada; [7]Pharmaceutical Outcomes and Policy Innovations Programme, Division of Translational Therapeutics, Department of Pediatrics, University of British Columbia, Vancouver, Canada

Corresponding Author: uamstutz@popi.ubc.ca

Conflict of Interest: None declared

Background: Anthracycline-induced cardiotoxicity (ACT) is a serious adverse drug reaction limiting its use and causing substantial morbidity and mortality in childhood cancer survivors. High cumulative doses are known to increase cardiotoxicity risk, but genetic factors are suspected to be important as well given the high inter-individual variability in tolerated doses.

Objective: Our aim was to identify genetic variants associated with ACT in patients treated for childhood cancer.

Methods: We carried out a genetic association study using 2977 single nucleotide polymorphisms (SNPs) in 220 key drug biotransformation genes in a discovery cohort of 156 anthracycline-treated children from British Columbia, with replication in a second cohort of 188 pediatric oncology patients from across Canada as part of the Canadian Pharmacogenomics Network for Drug Safety (CPNDS).

Results: We identified a highly significant association of a coding variant in a transporter gene with ACT (P=1.0x10^{-4}). We found further evidence (P<0.01) for associations with risk and protective variants in other genes including several other transporters. Combining these variants with important clinical risk factors in a predictive model, we classified patients into three risk groups. In the high-risk group, 75% of patients were accurately predicted to develop ACT, with 36% developing ACT within the first year; whereas in the low-risk group, 96% of patients were accurately predicted not to develop ACT.

Conclusions: We have identified multiple genetic variants associated with ACT. Combined with clinical risk factors, genetic risk profiling can be

used to identify high-risk patients who can then be provided with safer treatment options.

171. Study of Natural Health Product Adverse Reactions (SONAR): Piloting an Active Surveillance Model in Community Pharmacies

Vohra S[1,2,3], Cvijovic K[4,5], Boon H[4], Foster B[6,7], Jaeger W[5], LeGatt D[8], Cembrowski G[8], Murty M7, Vu D7, Leitch R7, Tsuyuki R2,9,10 Barnes J[11], Charrois T[12], Arnason J[9], Ware M[13], Rosychuk R[14,15]. [1]CARE Program, Department of Pediatrics, Faculty of Medicine, University of Alberta, Edmonton, Alberta; [2]School of Public Health, University of Alberta, Edmonton, Alberta; [3]Department of Medicine, University of Alberta, Edmonton, Alberta; [4]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario; [5]Department of Clinical Pharmacy and Diagnostics, University of Vienna, Vienna, Austria; [6]Faculty of Medicine, University of Ottawa, Ottawa, Ontario; [7]Health Canada, Ottawa, Ontario; [8]Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta; [9]Department of Biology, Faculty of Science, University of Ottawa; [10]Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta; [11]School of Pharmacy, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand; [12]School of Pharmacy, Curtin University of Technology, Perth, Australia; [13]Research Institute, McGill University Health Centre; [14]Department of Pediatrics, and Department of Medicine, University of Alberta, Edmonton, Alberta; [15]Women and Children's Health Research Institute, Edmonton, Alberta
Corresponding Author: sunita.vohra@ualberta.ca
Conflict of Interest: None declared

Background: Many consumers use natural health products (NHPs) concurrently with prescription medications. As NHP-related harms are under-reported through passive surveillance, the safety of concurrent NHP-drug use remains unknown.
Objective: To assess the feasibility of active surveillance in participating community pharmacies to identify adverse events related to concurrent NHP-prescription drug use.
Methods: Participating pharmacists asked individuals picking up prescription medications about (i) concurrent NHP/drug use in the previous three months and (ii) the presence of potential adverse events. If a potential adverse event was identified and the patient agreed, a research pharmacist conducted a guided telephone interview to gather additional information.
Results: Over a total of 112 pharmacy weeks, 2615 patients were screened, of which 1037 (39.7%; 95% CI: 37.8% to 41.5%) reported concurrent NHP and prescription medication use. A total of 77 patients reported a possible AE (2.94%; 95% CI: 2.4% to 3.7%), which represents 7.4% of those using NHPs and prescription medications concurrently (95%CI: 6.0% to 9.2%).
Conclusion: Compared to passive surveillance, this study found active surveillance to markedly improve NHP adverse event reporting rates. Active surveillance is feasible and offers improved quantity and quality of adverse event data, allowing for meaningful adjudication to assess potential harms.

172. Adherence Measurement Methods among HIV Positive Adolescents in Uganda: A Prospective Cohort Pilot Study

Wiens MO[1, 2], MacLeod S[1, 2], Musiime V[3], Ssenyonga M[3], Kizza R[4], Kitaka S[5], Adome RO[5], Ssali F[3]. [1]University of British Columbia, Vancouver, BC; [2]Child and Family Research Institute, Vancouver, BC; [3]Joint Clinical Research Centre, Kampala, Uganda; [4]Norra Stockholms Psykiatri, St Görans Hospital, Stockholm, Sweden; [5]Makerere University, Kampala, BCUganda.
Correspnding Author: mowiens@interchange.ubc.ca
Conflict of Interest: None declared

Introduction: The quality of traditional adherence measurements among adolescents is difficult to assess. Antiretroviral (ARV) adherence research among adolescents living with HIV in resource-constrained countries is limited.
Objectives: Our primary objective was determining feasibility of a large-scale, long-term study using electronic adherence monitoring in Uganda. Our secondary objective was to compare accuracy of pill-count (PC) and self-report (SR) adherence with electronic medication vials (eCAPs). eCAP's record compliance in real-time with data downloaded during refills.
Methods: Adolescents receiving care at the Joint Clinical Research Centre in Kampala, Uganda were recruited. ARV's were dispensed in eCAPs for 1 year. Person-pill-days (one day where adherence

was measured for one medication in one person) were calculated for each patient and a weighted paired t-test was used to compare the levels of adherence among all subjects for three different adherence methods.
Results: Fifteen patients were included: 40% were female, mean age was 14, mean baseline CD4 count was 244, and average treatment duration was 9 months at study entry. 4721 person-pill-days were observed. Several eCAPs required replacement during the study resulting in some data loss. Consent rate was high (94%) but was slow due to age limit cut-points, indicating that a future study should be multi-site. A longitudinal examination of the eCAP data showed that while most non-adherence among individual subjects was time-dependant (during times of poor adherence all medications were affected), some was drug-dependant (adherent to one medication but not others). Our small pilot sample precluded a regression analysis to further explore this effect. Overall compliance for SR was 99%, PC was 97% and eCAP was 88% ($p<0.05$ for all comparisons). 93%, 67% and 23% of patients had a compliance of greater than 95% among SR, PC and eCAP methods, respectively.
Conclusions: A large-scale adherence study in Uganda is feasible using a more robust electronic monitoring system. Adherence measurements produced by pill counts and self reporting methods appear to overestimate adherence measured electronically. However, overall adherence measured with all methods was still clinically acceptable.

173. Determinants of CYP3A4 Expression and Activity in the Huh7 Human Hepatoma Cell Model of Non-Alcoholic Fatty Liver Disease

Woolsey SJ [1], Beaton MD[3], Tirona RG[1,2]. [1]Department of Physiology and Pharmacology; [2]Department of Medicine, Division of Clinical Pharmacology, [3]Division of Gastroenterology, Lawson Health Research Institute, The University of Western Ontario, London, Ontario, Canada
Corresponding Author: swoolsey@uwo.ca
Conflict of Interest: None declared

Background: The recent rise in rates of obesity, diabetes and metabolic syndrome among the Western population, has caused a parallel increase in the prevalence of non-alcoholic fatty liver disease (NAFLD). A defining characteristic of NAFLD is steatosis within hepatocytes. Despite the high incidence, little is known regarding the effect of NAFLD on hepatic drug metabolism and its impact on drug response. We hypothesize that hepatic CYP3A activity is altered as a result of fat accumulation, leading to changes in the pharmacokinetics of everyday drugs.
Objective: Establish an *in vitro* model of human NAFLD to study regulation of expression and activity of CYP3A4.
Methods: To better understand the mechanisms involved in the regulation of CYP3A4 in NAFLD, we will use the Huh7 human hepatoma cell line known to natively express CYP3A4. In the cell model, Huh7 cells are incubated with free fatty acids to induce fat overloading similar to that found in steatotic liver. Lipid quantification, cytotoxicity and CYP3A4 mRNA and protein expression will be examined. Furthermore, biotransformation activity will be assessed by measuring the metabolism of midazolam, a probe drug substrate for CYP3A4 enzymes.
Results: Preliminary data from our laboratory indicates that patients with NAFLD have altered CYP3A4 expression and activity.
Conclusions: It is expected that this in vitro model of human NAFLD will provide the platform for further studies aimed to define the signaling pathways involved in regulating CYP3A4 activity. Together with studies in patients, these studies may provide a basis for better and safer pharmacotherapy in NAFLD.

174. Hydrogen Sulfide Mediates the Inhibitory Effect of Sulforaphane on the Proliferation of Human Prostate Cancer Cells

Guangdong Yang[1], Yanxi Pei[2], Qiuhui Cao[3], Lingyun Wu[4], Rui Wang[3]. [1]School of Kinesiology, Lakehead University, Thunder Bay, Canada; [2]College of Life Science, Shanxi University, Taiyuan, China; [3]Department of Biology, Lakehead University, Thunder Bay, Canada; [4]Department of Pharmacology, University of Saskatchewan, Saskatoon, Canada
Corresponding Author: gyang@lakeheadu.ca
Conflict of Interest: None declared

Background: Hydrogen sulfide (H_2S) is a novel gasotransmitter that regulate cell proliferation and other cellular functions. Sulforaphane is a sulphur-containing compound that exhibits anticancer properties, and young sprouts of broccoli are

particularly rich in sulforaphane. There is consistent epidemiological evidence that the consumption of sulphur-containing vegetables, such as garlic and cruciferous vegetables, may help reduce the occurrence of prostate cancer.

Objectives: We want to determine whether H_2S mediates the anti-survival effect of sulforaphane on prostate cancer and the underlying mechanisms.

Methods: H_2S level, cell viability, and protein expression were measured by methylene blue method, MTT assay, and western blotting, respectively.

Results: A large amount of H_2S is released from sulforaphane when sulforaphane was injected into mice or added into the cell culture medium or mixed with mouse liver homogenates. Both sulforaphane and NaHS (a H_2S donor) decreased the viability of PC-3 cells (a human prostate cancer cell line) in a dose-dependent manner, and supplement of methemoglobin or oxidized glutathione (two H_2S scavengers) reversed sulforaphane-reduced cell viability. NaHS also significantly inhibited PC-3 cell migration. We further found both cystathionine gamma-lyase (CSE) and cystathionine beta-synthase are expressed in PC-3 cells and mouse prostate tissues. H_2S production in prostate tissues from CSE knockout mice was only 20% of that from wild-type mice, suggesting CSE is a major H_2S-producing enzyme in prostate. CSE overexpression enhanced H_2S production and inhibited cell viability in PC-3 cells. In addition, sulforaphane and NaHS activated p38 mitogen-activated protein kinases (MAPK) and c-Jun N-terminal kinase (JNK). Pre-treatment of PC-3 cells with methemoglobin decreased sulforaphane-stimulated MAPK activities. Suppression of both p38 MAPK and JNK reversed H_2S- or sulforaphane-reduced viability of PC-3 cells.

Conclusions: Our results demonstrated that H_2S mediates the inhibitory effect of sulforaphane on the proliferation of PC-3 cells, which suggests that H_2S-releasing diet or drug might be beneficial in the treatment of prostate cancer.

CC-CRS Posters - Day 2

Thursday, May 26, 2011

175. Synthesis, Characterization and Evaluation of Bone Targeting Salmon Calcitonin Analogues in Normal and Osteoporotic Rats

Krishna Hari Bhandari, Madhuri Newa, Jillian Chapman and *Michael R. Doschak. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada.

Purpose: To synthesize, characterize & evaluate the *in vivo* efficacy of novel bone-targeting salmon calcitonin (sCT) analogues, in order to assess the therapeutic efficacy of an antiresorptive drug with imparted bone targeting potential (using bisphosphonate [BP] conjugation) and an improved pharmacokinetic profile (using pegylation).
Methods: Bone targeting pegylated sCT (sCT-PEG-BP) was synthesized, characterized by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analysis and compared against non-pegylated bone targeting sCT analogue (sCT-BP) and unmodified, commercially available sCT. The effect of BP or PEG-BP upon sCT secondary structure was examined by Circular Dichroism and the sCT analogues were evaluated for *in vitro* bone mineral affinity and specificity using a binding assay for bone hydroxyapatite and several other calcium salts. Antibody epitope binding specificity of this analogue was also determined using enzyme-linked immunosorbent assay (ELISA), by reacting bone targeting sCT analogues with calcium phosphate coated Osteologic® plates and detecting the bound sCT using anti-sCT antibody. Potential cytotoxicity of sCT-PEG-BP was evaluated in monocytic RAW 264.7 cells, and sCT bioactivity and calcitonin receptor (CTR) binding potential was evaluated using an *in vitro* intracellular cAMP stimulation assay in human T47D breast cancer cells. Finally, *in vivo* efficacy of each compound was evaluated by determining the plasma level of calcium after sub-cu administration in normal rats, and in a rat model of Osteoporosis, secondary to ovariectomy (OVX). *In vivo* micro-computed tomography (micro-CT) was used to temporally map and quantify alterations in bone volume and bone mineral density (BMD) in the same animals at 1, 4, 8 and 12 weeks after OVX surgery. Sixteen 6 week old virgin female rats underwent OVX surgery followed by the daily sub cu injection of 2.5 IU/kg/day sCT or equivalent analogues, and compared to four sham-operated, placebo treated control rats.
Results: Our results showed the chemical coupling of PEG-BP or BP to sCT altered its secondary structure without altering its receptor and antibody binding ability. sCT analogues retained strong sCT bioactivity and CTR binding affinity, were non-toxic to RAW 264.7 cells in culture and elicited a comparable hypocalcemic effect (to that of unmodified sCT) after injection in normal rats. sCT-PEG-BP significantly outperformed unmodified sCT (marketed product) at the initial dose tested, preserving *in vivo* bone volume, BMD and trabecular micro-architecture in Osteoporotic rats.
Conclusion: Bisphosphonate-mediated targeting of pegylated sCT to bone greatly improved drug efficacy and represents a new class of targeted antiresorptive compounds that has not previously been attempted.

176. Encapsulation of P-Glycoprotein Inhibitors inside Polymeric Micelles Can Reduce Doxorubicin-Free Drug Interactions

Ziyad Binkhathlan[1], Anoosh Shayeganpour[1], Dion R. Brocks[1], and Afsaneh Lavasanifar[1,2]. [1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada; [2]Department of Chemical and Material Engineering, University of Alberta, Edmonton, Alberta, Canada

Purpose: Co-administration of P-glycoprotein inhibitors such as cyclosporine A (CyA) and its analogue, valspodar with doxorubicin (DOX) have resulted in an increased DOX toxicity, which is due to altered anticancer drug pharmacokinetics. The purpose of this study was to evaluate whether or not these adverse interactions could be avoided by

encapsulating the P-glycoprotein inhibitor (CyA or valspodar) inside polymeric micelles.
Methods: Block copolymers of methoxy PEO-*b*-PCL (methoxy PEO and PCL of 5000 and 13000 g/mol, respectively) were synthesized. CyA or valspodar was physically encapsulated in PEO-*b*-PCL micelles using cosolvent evaporation method. Control formulation was composed of CyA (as Sandimmune®) or valspodar dissolved in a mixture of Cremophor EL (CrEL) and ethanol. Sprague-Dawley rats (250-350 g) were used in this study (6 rats/group). The commercially available DOX (Adriamycin® PFS) was administered as a single dose of 5 mg/kg intravenously (iv) either alone or 30 minutes following a single i.v. dose (10 mg/kg) of either CyA or valspodar. Serial blood samples were collected. Plasma was assayed for DOX concentrations using an HPLC method. Non-compartmental approach was used to estimate the pharmacokinetic parameters of DOX.
Results: Co-administration of DOX with either Sandimmune® or valspodar (in the CrEL-based formulation) was associated with more than 50% reduction in DOX clearance (CL) ($p < 0.05$; ANOVA). Although there was nearly 40% reduction ($p < 0.05$) in the CL of DOX with the polymeric micellar formulation of CyA, there was only 6% reduction ($p > 0.05$) with the polymeric micellar formulation of valspodar. Consequently, there was more than a 100% increase ($p < 0.05$) in the DOX AUC associated with the co-administration of either Sandimmune® or valspodar. This was in line with a 70% increase ($p < 0.05$) in DOX AUC when given with CyA-loaded polymeric micelles. In contrast, there was no change ($p > 0.05$) detected in DOX AUC when administered with the valspodar polymeric micellar formulation. Although the $t_{1/2}$ of DOX was increased in all the test groups, it was statistically significant ($p < 0.05$) only in the case of valspodar in the CrEL formulation.
Conclusions: The results suggest that encapsulation of valspodar inside polymeric micelles could avoid the alteration of DOX pharmacokinetics in rat.

177. Melatonin and Resveratrol Increase Bone Accrual in Aging Rats

Hazem Eimar*, Isabel F. Tresguerres†, Rubens Albuquerque*, Jake Barralet*, Jesus A.F. Tresguerres‡, Faleh Tamimi*. *Faculty of Dentistry, McGill University, Montreal, Quebec, Canada; † School of Dentistry, Complutense University, Madrid, Spain; ‡ Faculty of Medicine, Complutense University, Madrid, Spain

Purpose: The aim of this study was to assess the improvement in bone accrual properties in rats treated with Melatonin, Growth Hormone (GH) & Melatonin, and Resveratrol.
Methods: Twenty male, 24-months-old Wistar rats were divided into four randomly assigned groups (n = 5) that were treated either with Melatonin, GH & Melatonin, or Resveratrol, while the fourth group was left as control. The animals were treated for 10 weeks before being sacrificed. The Femurs were dissected and fixed in formaldehyde. Then, micro-computed tomography (micro-CT) scans and histological analysis were made to compare bone architecture among the study groups. Moreover, bone mechanical properties were tested using the three-point bend testing method. The statistical significance ($P<0.05$) among treatment groups was calculated using Kruskal–Wallis statistical analysis with pair-wise comparison.
Results: Rats treated with Melatonin or Resveratrol demonstrated higher bone volume percentage than control group rats. Moreover, Resveratrol treated rats had a higher bone trabeculae per volume than the other study groups. On the other hand, GH & Melatonin treated rats were observed to have higher cortical bone thickness and stiffness than the other study groups.
Conclusions: Within the limitation of this animal study, administration of either melatonin or Resveratrol to old rats might improve trabecular bone accrual, while administration of GH & melatonin could improve cortical bone accrual as well as bone mechanical properties.

178. Antiresorptive Drug Delivery Strategy using Single Chain Fraction Variable as an Osteoclast Targeting Platform

Madhuri Newa, Krishna Hari Bhandari, Michael Lam, Rohit Kalvapalle, Mavanur Suresh, Michael R. Doschak. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada.

Purpose: Osteoclasts (OC) are multinucleated cells associated with bone resorption and primarily responsible for the pathogenesis of osteoporosis and osteoarthritis. None of the current antiresorptive therapies are designed to target osteoclasts directly in order to deliver an active drug cargo. As single

chain fraction variables (scFv) have exquisite target recognition specificity and are less immunogenic than full length antibody, we hypothesized that scFvs against Receptor Activator of Nuclear factor Kappa B receptors (RANK) found on OC would serve as a platform to selectively target and deliver conjugated antiresorptive drug cargo to OC. Salmon Calcitonin (sCT) was chosen as the model drug as calcitonin receptors are expressed on mature bone resorbing osteoclasts.

Methods: ScFv against RANK receptor was generated using phage display. RANK binding scFv clones were screened by ELISA and purified by Ni-NTA column followed by dialysis. Protein concentration was determined by Bicinchoninic protein assay. ScFv was characterized by SDS-PAGE, ELISA and Western Blot. To synthesize scFv-sCT bio-conjugates, thiol groups were generated in scFv using 2-Iminothiolane and reacted with sCT-PEG-MAL synthesised from sCT and NHS-PEG-MAL. Conjugates were purified by dialysis and characterised by SDS-PAGE, ELISA and Western Blot. Conjugate functionality will be confirmed by tartarate resistant acid phosphatase (TRAP) activity, TRAP staining, Resorption Pit assay , cyclic AMP assay and MTT assay. The scFv-sCT conjugate will further be assessed 'in-vitro' in osteoclast cell culture using reverse transcriptase polymerase chain reaction for mRNA expression of NFATc1 (a downstream gene activated by RANK stimulation). In vivo efficacy of conjugate will be tested in a rat model of Osteoporosis (secondary to ovariectomy, OVX). The pharmacokinetic biodistribution of subcutaneously injected conjugate will be studied by labelling the conjugate with Iodine-125.

Results: The results have been quite encouraging from generation of scFv against RANK receptor and its characterization by SDS-PAGE, ELISA and Western Blot. Antiresorptive hormone salmon calcitonin was successfully conjugated to this anti RANK scFV and characterised by SDS-PAGE, ELISA and Western Blot. Conjugate efficacy will be determined by in vitro and in vivo evaluation.

Conclusion: This antiresorptive drug delivery strategy using scFv as osteoclast targeting platform may prove of eventual utility in the therapeutic treatment of bone disease.

179. Injectable Thermosensitive Microgel-Hydrogel Composites for Drug Delivery

Daryl Sivakumaran, Danielle Maitland, and Todd Hoare. Department of Chemical Engineering, McMaster University, Hamilton, Ontario.

Purpose: Drug delivery systems are essential tools in the fight against disease. Challenges in development can part be addressed through the use of microgels, hydrogel particles with nanoscale dimensions. Spherical microgels made from poly(N-isopropylacrylamide) (PNIPAM)) are of particular interest given that the effective diameter and water content of these microgels decrease dramatically at ~32^{o}C. However, the nanoscale dimension of microgels typically results in relatively rapid drug release and permits rapid phagocytosis by the body, resulting in rapid clearance of the microgel from the injection site to the liver or spleen. To address this issue, microgels can be immobilized within an injectable hydrogel network which is a free-flowing liquid outside the body but quickly gels upon injection inside the body. In addition, the drug release rate from such systems may be engineered according to the crosslink density, swelling, and drug-polymer affinity of both the hydrogel and microgel phases.

Materials and Methods: The hydrogels were fabricated from carboxymethyl cellulose (CMC) and dextran modified with hydrazide (CMC A) and aldehyde (CMC B, Dex B) functional groups. When mixed via co-injection through a needle, these two polymers rapidly form a hydrazone-crosslinked hydrogel network and can encapsulate AA-NIPAM microgels inside the hydrogel network. Drug release from the composite hydrogels was analyzed using a transwell plate technique and quantified using UV/VIS spectrophotometry. Biocompatibility was assayed with the MTT assay, using fibroblasts and myoblasts as model cells.

Results: Current results show that the release of bupivacaine, a cationic local anesthetic, can be sustained over a period of up to 60 days using composite hydrogel systems. Release rates scaled directly with the anionic functional group content of the microgel phase; the higher the degree of acrylic acid functionalization, the higher the ionic binding between the cationic drug and the microgel and the slower the release. The composite hydrogels, hydrogel pre-polymers, and microgels all showed no significant cytotoxicity to fibroblasts or myoblasts at concentrations up to 2mg/mL according to the MTT

assay.
Conclusion: The greatest, and most predictable, variation in drug release is achieved through changing degree of functionalization of PNIPAM microgel used. Drug release durations of up to 60 days can be achieved using hydrogel-microgel composites, significantly longer durations of release than can typically be achieved using hydrogels or microgels alone. As the microgels and polymers are not cytotoxic, it is suggested that they may be used as an option for *in vivo* local drug delivery vehicles.

180. Celastrol-Encapsulated Hydroxy-Terminus Poly(amidoamine) Dendrimers Inhibit Lipopolysaccharide-Mediated Inflammatory Signaling in Microglia

Ghareb M. Soliman[1,2], Sebastien Boridy[1], and Dusica Maysinger[1]. [1] Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada, [2]Department of Chemistry, McGill University, Montreal, Quebec, Canada

Purpose: Celastrol is a quinone methide triterpene extracted from the root bark of *Trypterygium Wilfordii* (Chinese "Thunder God Vine") that has long been used in traditional Chinese medicine for the treatment of inflammatory diseases, autoimmune diseases, and cancer. However, celastrol's limited aqueous solubility and toxicity impede its clinical application. The objective of the present study was to encapsulate celastrol into both G4 PAMAM-NH_2 and G4 PAMAM-OH and test which of the two nano-delivery systems would be most effective at (1) solubilising celastrol; (2) reducing its cytotoxicity; and (3) increasing its anti-inflammatory potency.
Methods: Celastrol aqueous solubility was tested in the presence of different concentrations of G4-PAMAM-OH and G4-PAMAM-NH_2 dendrimers in PBS (pH 7.4). Anti-inflammatory activity of celastrol/PAMAM complexes was assessed in N9 microglial cells activated by lipopolysaccharide (LPS). Nitric oxide release from the cells was estimated by the Griess Reagent. The extent of MAPK (p38) phosphorylation inhibition in LPS-stimulated microglia in the presence and absence of celastrol alone and when incorporated into PAMAM dendrimers was also estimated.
Results: Celastrol aqueous solubility increased linearly with the concentration of PAMAM-OH and PAMAM-NH_2 due to electrostatic and hydrophobic interactions between the drug and dendrimer. Incorporation of celastrol into PAMAM-OH abolished the cytotoxicity observed with the drug alone or when incorporated into PAMAM-NH_2. Moreover, PAMAM-OH dose-dependently inhibited nitric oxide release on its own and to a greater extent with celastrol encapsulated. Celastrol-encapsulated PAMAM dendrimers inhibited LPS-induced phosphorylation of p38 supporting the link between p38 activation and NO release.
Conclusion: G4.0 PAMAM dendrimers greatly enhanced the aqueous solubility of celastrol and maintained its anti-inflammatory properties while completely abolishing the cytotoxicity caused by the drug alone.

181. Elemental Composition of Osteoporosis Rat Bone after Bisphosphonate and/or Strontium Ranelate Treatment by Electron Probe Micro Analysis

Yuchin Wu[1], Samer Adeeb[12], M. John Duke[3] and *Michael R. Doschak[13]. Dept. of Biomedical Engineering[1], Dept. of Civil Engineering[2], Faculty of Pharmacy & Pharmaceutical Sciences[3], University of Alberta, Edmonton, Alberta, Canada

Purpose: To explore the intensity and distribution compositions of certain elements such as Calcium (Ca), Strontium (Sr), Phosphorous (P) and Magnesium (Mg) of Osteoporosis (OP) rat bones after Bisphosphonate (BP) and/or Strontium Ranelate (SrR) drug administration.
Methods: Sixteen 6-month old female SD rats were ovariectomized (OVX), and divided into 4 groups (n=4/group); OVX-Control, OVX-RIS (Risedronate [BP] treated), OVX-SrR (Strontium Ranelate [Protos®] treated), OVX-RIS+SrR, and compared with Sham-operated controls (n=3). After 16 wk of treatment (RIS 0.06 mg/kg q3.5d; SrR 308mg/kg qd p.o), rats were euthanized and dissected lumbar vertebrae (L3 and L4) were dissected and cut in half in transverse and coronal directions individually. Electron probe micro-analysis (EPMA) was conducted using a CAMECA SX 100 electron probe to map and quantify the distribution of Ca, Sr and P. Quantitative analysis determined the wt% of bone elemental composition according to the distribution maps.
Results: EPMA mapped elemental Sr deposition to the periosteal surface of cortical bone (50~100 μm thick), and to endosteal trabecular surfaces (20 μm thick), as well as to both vertebral growth plates.

The atomic ratio of (Ca+Sr)/P were similar (~1.667) for all non-SrR treatment groups (including OP control), but significantly reduced with SrR treatment (2.4%~6.6%, $p<0.05$), indicating Sr incorporation into bone mineral as Sr-HA. BP did not change the atomic ratio of P:Ca and the concentration of Mg (0.47±0.06 wt%) remained consistent in all groups. Sr deposition was significantly reduced on endosteal trabecular surfaces in OVX-RIS+SrR. In OVX-RIS+SrR, P:(Ca+Sr) and P:Ca atomic ratios on regions we mentioned above were significant different ($p<0.05$) from other groups. This evidence suggested that SrR not only changed the structure of hydroxyapatite (HA) to Strontium-hydroxyapatite (Sr-HA) but also fluctuated the mineralization when combined treatment with BP.

Conclusion: Our EPMA findings confirmed that mineralized bone matrix is heterogeneous in material composition, particularly after SrR treatment, where the Sr atom serves as a surrogate for elemental Ca uptake and incorporation in remodeling bone. Bone modeling (i.e. formation) frequency and duration was significantly reduced in OVX-RIS dosed rats co-administered SrR, compared to OVX-SrR dosed rats, as evidenced by EPMA analysis of incorporated elemental Sr. Our study establishes the potential that Sr drugs offer for use as exquisite in vivo tracers of bone turnover.

182. In Vitro Adhesion Study of Mucoadhesive Polymers and Gastrointestinal Mucosa

J. Xu[1], X.T. Le[1], D.C. Bassett[2], J.E. Barralet[2] and M.G. Cerruti[1,2]. [1]Department of Materials Engineering and [2]Faculty of Dentistry, McGill University, Montreal, Quebec, Canada. jinke.xu@mail.mcgill.ca

Purpose: We are developing a drug delivery system for oral delivery of calcitonin. Mucoadhesive polymers have shown good promise to functionalise drug carriers for targeted and sustained drug delivery to mucosal surfaces, such as the intestine [1]. In this study we evaluate the mucoadhesive force between chitosan and alginate hydrogels and *ex-vivo* porcine intestinal mucosa.

Methods: 2% (w/v) medium molecular weight (MMW) and low molecular weight (LMW) chitosan were dissolved in 1.75 M acetic acid, followed by neutralization with 5M NaOH to form thin hydrogel films. Alginate hydrogels were prepared by crosslinking 2% (w/v) sodium alginate with 0.2 M $CaCl_2$. Hydrogels were formed on a piece of cloth, and then attached to the testing probe giving a surface area of 1 cm^2. Covalent grafting of 2% (w/v) chitosan and poly (acrylic acid) (PAA) was conducted by a two-step method: the stainless steel foil (1cm×1cm) was first immersed in a solution containing 0.1M *p*-phenylenediamine and 0.1M $NaNO_2$ dissolved in 10ml H_2SO_4 (0.25M) for 3 hours. Iron powder was added as a reducing agent. The foil was removed to 0.5M $NaNO_2$ in 5ml H_2SO_4 (0.25M) and immersed for 60s. 2% (w/v) MMW chitosan and PAA were dropped on the surface allowing polymer molecules to be covalently bonded. Surface characterization of covalent grafting was examined by XPS. Force of adhesion of the hydrogels and *ex-vivo* porcine intestinal mucosa was measured using an Instron 5544 mechanical testing instrument equipped with a 100N load cell as shown in Figure 1.

Results: The chitosan and alginate hydrogels formed within a cloth matrix were easy to handle and place on the test probe. In air, the highest maximum detachment force (0.140 ± 0.030 N/cm^2) was measured with MMW chitosan, compared with 0.129 ± 0.020 N/cm^2 for chitosan LMW and 0.108 ± 0.015 N/cm^2 for alginate gels (Figure 2). XPS confirmed the covalent bonding of MMW chitosan and PAA on stainless steel. Tests of adhesion force using covalently grafted samples are in progress.

Conclusion: A setup for testing mucoadhesive properties was successfully developed. Mucoadhesive hydrogels were successfully synthesised and the mechanical testing procedure was validated. MMW chitosan was found to have the highest mucoadhesion out of the hydrogels tested in air. These results will contribute to the development of new oral drug delivery systems.

Reference: [1] J.D. Smart. The basics and underlying mechanisms of mucoadhesion. Advanced Drug Delivery Reviews 57 (2005) 1556-1568.

NHPRS Posters - Day 2

Thursday, May 26, 2011

Poster Session 3

183. Short Term Exposure of Adult and Juvenile Mice to a Proprietary Extract of North American Ginseng Results in Long Term, Quantitative Changes in the Hemopoietic and Immune Cells of the Blood, Spleen and Bone Marrow (NHPRS-P3_01)

Sandra C. Miller[1], Lianping Ti[1], Jacqueline J. Shan[2]. [1]Department of Anatomy & Cell Biology, McGill University, Montreal, QC, Canada; [2]Afexa Life Sciences, Inc., Edmonton, AB, Canada

The effects of daily dietary administration of CVT-E002, a proprietary extract of North American ginseng (Afexa Life Sciences, Inc., Edmonton, AB, Canada), on the hemopoietic and immune cells of the spleen, bone marrow and blood of mice was assessed in vivo. The extract was given daily in the chow to young, adult mice for one mo. immediately following which one group was euthanized and the hemopoietic and immune cells of their spleen, bone marrow and blood were assayed for CVT-E002-mediated alterations in 5 cell lineages (lymphocytes, monocytes, nucleated erythroid cells, granulocytes - mature and precursors). Another group of these mice was left for a subsequent 2 mo. on the control diet, following which the same organs were extracted and analyzed for these 5 cells types. In another study, juvenile mice, immediately upon weaning (age: 4 wk), were fed for one mo. with CVT-E002-containing chow immediately following which they were placed on control chow for the next 2 mo. At this time, their organs were extracted, and their cell types analyzed as above. The results revealed that CVT-E002 had a long-lasting, positive, quantitative effect on the lymphocytes and monocytes, regardless of age at commencement of CVT-E002. Granulocytes were variably affected in an age and/or organ-dependent manner whereas erythroid cells were unaffected. CVT-E002 appears, therefore, to imbue a profound, prophylactic effect on the immune system.

184. Lipid-Lowering Effects of Dilexaponantm (LIP-01), A Proprietary Formulation Consisting of *Coptis chinensis* and *Ilex kudingcha* Extracts (NHPRS-P3-02)

Richard Z. Lewanczuki[1], Jay Udani[2], Vinti Goel[3], Kelly Narine[3], Lei Ling[3], Sharla K. Sutherland[3], and Jacqueline J. Shan[3]. [1]University of Alberta, Edmonton, Alberta, Canada, [2]University of California, Los Angeles, USA, [3]Afexa Life Sciences Inc., Edmonton, Alberta, Canada.

Dilexaponan, a unique formulation comprised of proprietary polymolecular compounds extracted from Ilex kudingcha (Ku Ding Cha) and *Coptis chinensis* (Chinese Goldenthread), has been shown to reduce the secretion of Very Low Density Lipoproteins (vLDL) from human hepatoma cells, HepG2, in vitro. The combination of the extracts demonstrated a synergistic effect. The effects of Dilexaponan on plasma and liver lipid concentrations of rats were examined in four groups of male Zucker fatty (fa/fa) rats fed a high fat, high cholesterol diet. Three groups received daily oral administration of Dilexaponan (200, 400 or 800 mg/kg) and the fourth group received water. Animals were sacrificed four weeks after treatment and blood and liver samples were collected. Ratios of total:HDL and LDL:HDL cholesterol were dose-dependently reduced ($p<0.05$) in Dilexaponan-treated animals compared to controls. Furthermore, HDL cholesterol was increased by up to 40 % from baseline ($p<0.05$) at the 400 mg/kg dose compared to controls. Acute toxicity tests carried out in healthy Sprague-Dawley rats showed no adverse effects at single doses up to 5000 mg/kg. Subsequently, a pilot open-label dose-finding clinical trial was conducted to determine the safety and efficacy of chronic dosing of Dilexaponan in treating hypercholesterolemia in patients at low-risk of developing cardiovascular disease (Framingham risk score). The results of the first arm of the study (1 gram daily of Dilexaponan) showed significant decreases in LDL cholesterol levels after 2 and 3 months of treatment ($p<0.05$) and significant decreases in the ratios of total:HDL and LDL:HDL

cholesterol after one, two and three months of treatment ($p<0.05$). No increase in HDL levels was observed in this first arm of the clinical trial. These data demonstrate the safety and lipid-lowering effects of Dilexaponan and its potential application in cholesterol management. Future studies aim to develop a lipid-modulating formulation that both lowers vLDL and LDL levels and raises HDL levels in the clinical setting, thereby providing a more complete cholesterol management formulation.

185. Determination of Main Ginsenoside Contents in American and Chinese Ginsengs using NMR Spectroscopy (NHPRS-P3-03)

Huang Yawei[1], Wang Jiahua[1], Ting Wong[2], Jacqueline J. Shan[2], Lei Ling[2], Han Dongai[1]. [1]College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, China. [2]Afexa Life Sciences Inc., Edmonton, Canada.

Ginsenosides Rg1, Re, Rb1, Rc and Rd are common chemical components present in both Chinese and North American ginsengs, although to characteristically different extents. Quantitation of the contents is commonly performed with RP-HPLC-UV methods using commercially available standards. The assays are, however, time-consuming and can only be conducted in a laboratory environment. In this study, the application of near-infrared spectroscopy (NIR) is being investigated for potential on-site determination in fast screening of raw materials. The NIR method is built by spectra collected with a set of 75 Chinese and North American ginseng samples. The data treatment method employed, as well as how well the resultant OSC-PLS (orthogonal signal correction – partial least square) model can duplicate the primary HPLC method in main ginsenosides quantitation, will be discussed.

186. Immune Stimulating Effects of Immunity-FX™ in Cultured Cells (NHPRS-P3-04)

Meiqi Wang, Vinti Goel, Shaozao Wang, Lei Ling, Jacqueline J Shan. Afexa Life Sciences Inc., Edmonton, AB, Canada. Email: mwang@afexa.com

Immunity-FX, a new proprietary product of Afexa Life Sciences, consists of a mixture of CVT-E002 and an extract of *Ganoderma lucidum.* Using a mouse peritoneal macrophage cell line, RAW 264.7, an *in vitro* study was carried out to determine immune-modulating effects of Immunity-FX and its individual components. IMMUNITY-FX, CVT-E002 and the Ganoderma extract, all increased IL-1β and TNF-α production from the macrophage cells in a dose-related manner. To determine whether Toll-like receptor (TLR) activity may be involved in the immune stimulating effects of these extracts, a human embryonic kidney cell line (HEK 293) was used. These cells were transfected to express either TLR-2 or -4. CVT-E002 was found to enhance primarily TLR-2 mediated IL-8 secretion whereas the ganoderma extract was found to stimulate TLR-4 mediated IL-8 secretion. IMMUNITY –FX was found to enhance both TLR-2 and -4 mediated cytokine release, thus conferring potential protection from both gram positive and gram negative related bacterial infections. The results suggest that IMMUNITY-FX may have broader prophylactic effects against bacterial or viral diseases than either CVT-E002 or ganoderma extracts alone.

187. W9, A Medicinal Plant from the Pharmacopeia of the Eastern James Bay Cree, Exhibits Anti-diabetic Activities in Two Mouse Model of Diabetes (NHPRS-P3-05)

Hoda M. Eid [1,3,4], Meriem Ouchfoun [1,3,4], Antoine Brault [1,3,4], Diane Vallerand [1,3,4], Lina Mussalam [1,3,4], John T. Arnason [2,4], , Pierre S. Haddad [1,3,4]. [1]Natural Health Products and Metabolic Diseases Laboratory, Dept. of Pharmacology, Université de Montréal, Montreal, QC; [2]Phytochemistry, Medicinal Plant and Ethnopharmacology Laboratory, Dept. of Biology, University of Ottawa, Ottawa, ON; [3]The Institute of Nutraceuticals and Functional Foods (INAF), [4]Canadian Institutes of Health Research Team in Aboriginal Antidiabetic Medicines and Montreal Diabetes Research Center.

Aboriginal populations are particularly at risk for developing type 2 diabetes mellitus and its complications. In Canada, diabetes prevalence for these populations is at least three times higher than that of the general population. W9 has been identified among species used by the Cree of Eeyou Istchii of northern Quebec to treat symptoms of diabetes. In a previous study, the ethanol extract of W9 enhanced glucose uptake in C2C12 muscle cells via stimulation of AMP-activated protein kinase (AMPK) pathway. In this study, we investigated the

in vivo effect of this plant in two mouse models of type 2 diabetes. In the first one, KKA^y mice received W9 extract in drinking water (1%) for 10 days. In the second model, C57BL/6 mice were fed a high fat diet (HFD; ~35% lipids) for 8 weeks until they became obese and insulin resistant (diet-induced-obesity; DIO). Treatment then began by adding W9 extract to HFD at 3 different concentration (125, 250 and 500 mg/Kg) for another 8 weeks. In both models, W9 significantly decreased glycemia, strongly tended to decrease insulin levels, and this was accompanied with reduced fluid intake in the KKA^y model. This correlated with either a tendency or a frank increase in GLUT4 content and activation of the AMPK and/or Akt pathways in skeletal muscle. W9 treatment also improved hepatic steatosis by decreasing hepatic triglyceride levels and significantly activating the AMPK and Akt pathways. The results of the present study confirm that W9 represents a culturally relevant treatment option for Cree diabetics.

188. *AD01*, An Antidiabetic Plant of the Eastern James Bay Cree, Attenuates Insulin Resistance in a Diet-induced Obesity Mouse Model (NHPRS-P3-06)

Meriem Ouchfoun[1,3], Antoine Brault[1,3], Diane Vallerand[1,3], Lina Musallam[1,3], John T. Arnason[2,3], Pierre S. Haddad[1,3]. [1]Natural Health Products and Metabolic Diseases Laboratory, Dept. of Pharmacology, Université de Montréal, Montréal, Québec, Canada; [2]Phytochemistry, Medicinal Plant and Ethnopharmacology *Laboratory*, Dept. of Biology, University of Ottawa, Ottawa, Ontario, Canada; [3]Canadian Institutes of Health Research Team in Aboriginal Antidiabetic Medicines and Montreal Diabetes Research Center

We investigated the anti-diabetic effect of a medicinal plant used by the Cree nation of northern Quebec, *AD01*, in a diet-induced obesity (DIO) mouse model. C57BL6 mice were divided into 5 groups and given a standard CHOW diet (~ 4% of lipids) or a high fat-diet (~ 35% of lipids) during 8 weeks until they became obese and insulin resistant. Treatment then began by adding the plant extract at 3 different doses (125, 250, 500 mg/kg) into the high fat diet for another 8 weeks. At the end of the study, insulin sensitive tissues (liver, skeletal muscle, adipose tissue) were collected to investigate the plant's molecular mechanisms. *AD01* prevented weight gain (by 6%), reduced blood glucose (by 13%) and plasma insulin (by 65%) while preventing hepatic steatosis (up to 42% reduction in hepatic triglyceride levels) in DIO mice. Western immunoblot analysis demonstrated that *AD01* stimulated the activation of insulin dependent AKT pathway and increased the expression of Glut 4 in skeletal muscle. In the liver, *AD01* stimulated two pathways; the insulin dependent Akt and the insulin independent AMPK ones. The improvement of hepatic steatosis observed in DIO treated mice was associated with a reduction of inflammation and with a decrease in the hepatic content of SREBP-1. These data suggest that *AD01* exerts potential anti-diabetic action by improving insulin sensitivity and mitigating high-fat diet-induced obesity and hyperglycemia. They also validate the safety and efficacy of this plant. *AD01* thus represents a promising candidate for culturally relevant complementary treatment in Cree diabetics.

Poster Session 4

189. Risk-Based Supplier Quality Assurance Program For NHP & Dietary Supplement Manufacturers (NHPRS-P4-01)

Rebecca Robertson, Richard Vurdela. BCIT Technology Centre, Burnaby, British Columbia, Canada. Email: rebecca_robertson@bcit.ca

NHP & Dietary Supplement manufacturers must establish the identity of all functional raw materials to meet current regulatory GMP requirements. The methods used for identification must be appropriate for their intended use and scientifically valid. While identification of intact materials is relatively simple, processed material identification is more difficult because there are fewer fit for purpose analytical methods available. This problem is greatly magnified for those manufacturers purchasing hundreds of ingredients. A risk-based Supplier Quality Assurance program is a potent tool for prioritising the allocation of resources. The basis of this program is to assign risk-ratings to both raw materials and suppliers using risk matrices. The raw materials matrix includes criteria such as their intrinsic risks plus those specific to the manufacturer including the number of finished product dependencies. The supplier matrix includes criteria

such as supplier type and the geopolitical environment. The risk ratings are used to create a transparent, auditable method for demonstrating the prioritization of method development for raw material identification, the logic for matching raw materials to suppliers, establishing the stringency of raw materials monitoring programs and identifying which suppliers to audit.

190. Elemental Fingerprinting of *Panax quinquefolius* by Total Reflection X-Ray Fluorescence with Multivariate Data Analysis (NHPRS-P4-02)

Paula N. Brown[1], Wade Hurlburt[1,2], Ronan Yu[1], Jamie Finley[1], Lori Paley[1]. [1]Natural Health & Food Product Research Group, Centre for Applied Research & Innovation, BC Institute of Technology, Burnaby, BC, Canada; [2]Chemical & Environmental Technology, School of Energy, BC Institute of Technology, Burnaby, BC, Canada.
Email: Paula_Brown@bcit.ca

Panax quinquefolius L. (American Ginseng), an important medicinal crop, has been cultivated in Canada, the US and more recently China. Based on the US Ginseng Harvest Labeling Act of 2007, Ginseng was added to the 2008 Farm Bill that became the Final Rule on Mandatory Country of Origin Labeling on March 16th, 2009. This Rule requires retailers to label covered commodities offered for sale with the country/countries of origin. The ability to substantiate claims based on origin would serve to mitigate fraud and support Provenance marketing. To determine the feasibility of employing elemental fingerprints to establish the origins, American ginseng was obtained from three different countries for analysis. While inductively coupled plasma mass spectrometry (ICP-MS) is considered to be the industry standard for multi-element determination, total-reflection x-ray fluorescence (T-XRF) provides an alternative for trace level (ppb to ppm) microanalysis of both liquids and solids. For analysis by T-XRF all samples were prepared in quadruplicate, ground to ≤ 60 mesh, suspended on quartz discs using a 1% Triton X-100 solution, and analysed employing a gallium internal standard (2.5 ppm) for a 750 second acquisition time. A subset of samples were analysed for trace elements (<25 ppm) by ICP-MS as an orthogonal technique. All samples were analysed by a validated high performance liquid chromatography method for determining ginsenoside content for comparative purposes. Different statistical tools were applied to the multi-dimensional data set to visual clustering, evaluate variance and identify key contributing factors for determining provenance of American ginseng.

191. Using NMR-Based Metabolomics to Describe North American *Crataegus* species (NHPRS-P4-03)

Jennifer E. Edwards[1], Paula N. Brown[2], Nadia Talent[3], Timothy A. Dickinson[3], Paul R. Shipley[1]. [1]Department of Chemistry, University of British Columbia Okanagan, Kelowna, British Columbia, Canada; [2]BCIT Technology Centre, Burnaby, British Columbia, Canada; [3]Natural History, Royal Ontario Museum, Toronto, Ontario, and Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada.
Email: paul.shipley@ubc.ca

Hawthorn products are currently marketed in a number of North American, Asian, and European countries as an alternative treatment for hypertension, angina, arrhythmia, and the early stages of congestive heart failure. The use of hawthorn for the treatment of heart ailments dates back to the late 1800s, and numerous laboratory tests and clinical trials have demonstrated its efficacy. The chemistry of a number of hawthorn species has been studied extensively, including the quantification of sugars, flavonoids, anthocyanins, and volatile compounds. However, most of the available data pertains to those species that are native to Europe and Asia and comparatively little is known about the North American Crataegus species. With this in mind, NMR spectrometry was used to generate metabolic fingerprints of berry samples from three North American hawthorn species, including Crataegus jackii, C. douglasii, and a black-fruited taxon that generally resembles Crataegus cerrones. Metabolic fingerprinting and multivariate analysis was used to determine whether the berries from these species varied significantly from each other and multiple years worth of samples were collected to determine whether there was significant variation in berry metabolites from one year to the next. Berries from the two black-fruited species (C. douglasii and C. cerrones) were also collected at multiple points throughout the growing season to compare metabolite production at different stages of

ripeness. Principal component analysis was used to determine which chemical shift values, and therefore which chemical components, are the primary determinants of variation among berries from different species, seasons, and developmental stages.

192. A Bioanalytical Framework to Assess an Effect-Based Dose Measure of Anthraquinones in Rhei rhizoma Extract (NHPRS-P4-04)

Zeyad Alehaideb[1], Kimberly C Chin[1], Mei Cun Yao[2] and Francis CP Law[1]. [1]Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. Canada; [2]School of Pharmaceutical Sciences, Sun Yat-Sen University, University City Guangzhou, Guangzhou, China. Email: zia1@sfu.ca

A bioanalytical framework was developed for predicting the biologically effective dose of anthraquinone (AQ) bioactive in *Rhei rhizoma* (RR, *Rheum officinale* Baill.,) extracts. The proposed framework consisted of two steps: (a) determining *in vitro* cytochrome P450 1A2/3A4 (CYP1A2/3A4) inhibitory potency (IC_{50}) using the whole RR extract or individual AQ bioactive as an inhibitor, and (b) predicting the AQ effective dose using the whole mixture (WM) and individual component (IC) approaches of chemical mixture assessment. The AQ effective dose was expressed in mg equivalents aloe-emodin (AEM)/g RR, a measure of the amount of AEM required to eliciting the same IC_{50} as the unknown AQ mixture in the extract. Results of the study showed that nearly identical AQ effective dose measures were predicted by the CYP1A2-based WM and IC approaches indicating five major AQ congeners contributed additively to the overall inhibitory effect of the RR extract. In contrast, the WM and IC approaches predicted a different AQ effective dose measure in the CYP3A4 study probably because of the broad substrate specificity of the CYP3A4 isozyme. However, the average of the IC and WM effective dose measure was exceedingly similar in both CYP3A4 and CYP1A2 studies. The proposed framework provides the badly needed information for assessing the risks of AQ chemical mixture after RR administration *e.g.,* predicting the potential of RR/drug metabolic interaction *in vivo*. The framework as described in the present study also is applicable to studies of dietary supplements, nutraceuticals, and functional foods.

193. The Effects of *Rhodiola rosea* in Mediating Various Cytochrome Isozymes in the Metabolism of the Anti-Diabetes Medication, Repaglinide (NHPRS-P4-05)

Dania Alkhani[1], Rui Liu[1], Anthony Krantis[1], J. Thor Arnason[1], Brian C. Foster[1,2]. [1]Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, Ottawa, ON, Canada; [2]Therapeutic Products Directorate, Health Canada, Ottawa, ON, Canada.

Background: *Rhodiola rosea*, roseroot, is a perennial plant used for its "stimulatory" effects to treat weakness, depression and fatigue. Current uses for treatment of diabetes, tumor inhibition, hypothyrodism, anti-inflammation, and sexual dysfunction may increase the risk for adverse events (AE).
Purpose: This study is to determine the potential for Rhodiola to affect the safety and efficacy of the diabetes medication, Repaglinide.
Methods: Six formulated Rhodiola and 3 bulk (Alberta, Nunavik, and Siberia) samples were examined. Water, ethanol, and methanol extracts (5 mg/ml) were prepared fresh to examine their effects on human recombinant CYP 3A4 and liver microsomes (HLM).
Results: Our data indicates that the different Rhodiola extracts caused various degree of inhibition on human recombinant 3A4 (0 to 63.2%), on HLM (0 to 46.4%), and on HLM when given in conjunction with Repaglinide (2.9% - 59.1%). Phytochemical analysis determined that the extracts have differing chemical compositions, primarily of the active compounds salidroside and chemicals found under the class of rosavins.
Conclusions: With the rise in use of Rhodiola, these products may be administered concurrently with other health products. If Rhodiola has a considerable degree of inhibition on CYP 3A4, as well as other metabolic isozymes, then drugs given in conjunction with this natural medication can accumulate in the body to toxic levels causing adverse effects in patients. The study of the effect of Rhodiola on different metabolic enzymes when administered alone or in conjunction with Repaglinide serves as a valuable study to prevent adverse drug-drug interactions.

194. Determination of Rhaponticin Activity on CYP2C9, 2C19 and 3A4 *in vitro* (NHPRS-P4-06)

Sheila Smiley[1], Rui Liu[1], J. Thor Arnason[1], Brian C. Foster[1,2]. [1]Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, Ottawa, ON, Canada; [2]Therapeutic Products Directorate, Health Canada, Ottawa, ON, Canada.

Background: Rhaponticin (*Larix laricina*) has long been used for a variety of therapeutic purposes. Its aglycone, rhapontigenin, is believed to be the active form of the compound. Gliclazide, an anti-diabetic drug, is metabolized primarily by CYP 2C9 and 2C19. Rhapontigenin may interfere with gliclazide metabolism through inhibition of these cytochromes.
Purpose: This study looks to determine the effects of rhaponticin and rhapontigenin on various cytochrome P450s and gliclazide metabolism.
Methods: Rhapotigenin was obtained by β-glucosidase hydrolysis of rhaponticin. Both compounds along with gliclazide were prepared into 100% methanol extracts (1mg/ml). All compounds were studied for their inhibitory effects on CYP 2C9, 2C19 and 3A4 using microtiter fluorometric assays. The concentrations for the compounds used in the assays were 5µg/ml and 10µg/ml for 2C9/2C19 and 3A4 inhibition, respectively. Additionally, rhapontigenin was examined at varying concentrations, from 0.078µg/ml to 5 or 10µg/ml.
Results: Gliclazide showed no significant inhibition of any of the isozymes. Rhaponticin did not show CYP 2C9 or 3A4 inhibition, however 17.2% inhibition of CYP 2C19 was seen. Meanwhile, it was determined that rhapontigenin had IC_{50} values of 2.7µM (0.7µg/ml), 7µM (1.8µg/ml) and 30µM (7.7µg/ml) for CYP 2C9, 2C19 and 3A4, respectively.
Conclusions: As an inhibitior of gliclazide metabolism, rhapontigenin is expected to interact with the drug. Further studies are needed to determine how rhaponticin and rhapontigenin affect the metabolic profile of gliclazide.

Registrants (as of May 5, 2011)

Abdulla, Dalya	Sheridan College
Adomat, Hans	Vancouver Prostate Centre
Aghazadeh-Habashi, Ali	University of Alberta
Ahmed, Fida	University of Ottawa
AlAghawani, Wael	International Uuniversity for Science and Education
Aldawsari, Fahad	University of Alberta
Alexander, Kenneth	The University of Toledo
Aljudaibi, Bandar	London Health Science Centre
Alshamsan, Aws	King Saud University
Amstutz, Ursula	University of British Columbia
Aoki, Fred	University of Manitoba
Armstrong, Catherine	CRCHUM-Université de Montréal
Arnason, John	University of Ottawa
Badea, Ildiko	University of Saskatchewan
Balasuriya, Nileeka	Nova Scotia Agricultural College
Balneaves, Lynda	University of British Columbia
Barghian, Mehrdad	Quality Compliance Labs
Barl, Branka	NRC - IRAP
Barralet, Jake	McGill University
Barrett, Brendan	Memorial University of Newfoundland
Bassett, David	McGill University
Bayomi, Mohsen	King Saud University
Bellemore, Julie	Health Canada
Ben Abdennebi, Amine	Université de Montréal
Bendayan, Reina	University of Toronto
Bennett, Brian	Queen's University
Bennett, Stephanie	University of Ottawa
Berger, Jean Luc	World Courier
Berger, Neil	Pharma Medica Research
Bernatchez, Pascal	University of British Columbia/St. Paul
Bertrand, Nicolas	University of Montreal
Betancourt, Aimesther	Corealis Pharma
Bhandari, Krishna	University of Alberta
Blais, Martine	Centre Hospitalier Universitaire Sainte-Justine
Boch, Ron	Variation Biotechnologies Inc.
Bodapati, Krishna	University of Alberta
Boivin, Taryn	Cardiome Pharma Corp
Bourassa, Pierre	NSERC - Montreal
Brault, Antoine	CIHR Team in Aboriginal AntiDiabetic Medecines
Brien, James	Queen's University
Brocks, Dion	University of Alberta
Burger, Dylan	Kidney Research Centre
Burrill, Wendy	Afexa Life Sciences
Burt, Helen	University of British Columbia
Buschmann, Michael	Ecole Polytechnique
Buxton, Ian	Saxon Pharmaceutical Consulting
Cai, Ye	Afexa Life Sciences
Calon, Frederic	Université Laval
Campbell, Scott	McMaster University
Carey, Nathalie	Hospital for Sick Children
Carleton, Bruce	University of British Columbia
Carnevale, Amanda	Hospital for Sick Children
Castelan, Osvaldo	Instituto Politécnico
Cerruti, Marta	McGill University
Charbel, Chimène	Montreal Heart Insititute
Chatenet, David	Institut National de la Recherche Scientifique
Chaudry, Shahnaz	Hospital for Sick Children
Chavhan, Sandip	University of Baroda
Chemali, Raja	McGill University
Cheung, Richard	Fasken Martineau DuMoulin LLP
Chochkeh, Madona	University of Windsor
Chouinard, Guy	McGill University
Chouinard, Annie	CRBM
Chu, Stephanie	University of Manitoba
Chuang, Andrew	University of Toronto
Cieniak, Carolina	University of Ottawa
Clark, Deidre	Memorial University of Newfoundland
Cockerline, Carla	Nutrasource Diagnostics Inc.
Colinge, Jacques	CeMM-Research Center for Molecular Medicine GmbH
Colson, Kimberly	Bruker BioSpin
Colucci, Philippe	University of Montreal
Coventry, Krista	Nutrasource Diagnostics Inc.
Cuerrier, Alain	Jardin botanique de Montreal
Cumbleton, Anita	University of Alberta
Damha, Masad	McGill University

Davidovits, Judith	Hospital for Sick Children
De Paula, Marcio	McGill University
Deb, Subrata	The Vancouver Prostate Centre
DeGorter, Marianne	University of Western Ontario
DeMarte, Josie	Boehringer Ingelheim Canada Ltd
Dentali, Steven	American Herbal Products Assoc
Desmarais, Julie	McGill University Health Centre
Ding, Weimeng	Ecole Polytechnique de Montreal
Dionne, Jean-Yves	Apothicaire-Consultant
Doillon, Charles	Laval University
Doschak, Michael	University of Alberta
Dresser, George	London Health Sciences Centre
du Souich, Patrick	Université de Montréal
Edwards, Jennifer	University of British Columbia
Eimar, Hazem	McGill University
El-Aneed, Anas	University of Saskatchewan
El-Bagory, Ibrahim	King Saud University
El-Khoury, Nabil	University of Montreal
Elzagallaai, Abdelbaset	University of Western Ontario
Endrenyi, Laszlo	University of Toronto
Facecchia, Katie	University of Windsor
Fakhoury, Johans	McGill University
Fakhoury, Marc	Mcgill University
Fediuk, Daryl	University of Manitoba
Feng, Qingping	Unversity of Western Ontario
Flockhart, David	Indiana University School of Medicine
Ford, Debra	Sheffield Bio-Science
Foster, Brian	Health Canada
Francis, Mira	Algorithme Pharma
Fujii, Hisaki	Hospital for Sick Children
Gabathuler, Reinhard	biOasis Technologies Inc.
Gabr, Raniah	University of Alberta
Gagnon, Daniel	Herbs Etc.
Gallouedec, Francois	FMC BioPolymer
García-Pérez, Martha-Estrella	Université Laval
Garrioch, Colin	Nutrasource Diagnostics
Gaudreault, France	Santé Canada
Gershkovich, Pavel	University of British Columbia
Gijsen, Violette	Hospital for Sick Children
Gilzad Kohan, M.Hossein	University of Alberta
Giroux Portelance, Simon	Université de Montréal
Gobeil, Fernand Junior	Université de Sherbrooke
Godin, Richard	Université de Montréal
Goel, Vinti	Afexa Life Sciences
Gong, Inna	University of Western Ontario
Goni, Vijay	Post Graduate Institute of Medical Education
Gosselin, Patrick	Corealis Pharma
Grant, Denis	University of Toronto
Gros, Robert	Robarts Research Institute
Grove, Geoffrey	Sotax (Canada) Ltd.
Gu, Xiaochen	University of Manitoba
Guirguis, Micheal	Alberta Blue Cross
Gunness, Patrina	Hospital for Sick Children
Haddad, Pierre S	Université de Montréal
Haddadi, Azita	University of Saskatchewan
Hafsat, Shittu	Université de Montréal
Hamel, David	Université de Montreal
Hamet, Pavel	Centre de Recherche - Centre Hospitalier
Hanly, Lauren	University of Western Ontario
Harbilas, Despina	Université de Montréal
He, Juan	BioPharma Services
Hegele, Robert	Robarts Research Institute
Heyes, James	Tekmira Pharmaceuticals
Hicks, Joshua	Bruker BioSpin
Hitzler, Johann	Hospital for Sick Children
Hizel, Candan	University Marmara & Yeditepe University
Ho, Emmanuel	University of Manitoba
Hoare, Todd	McMaster University
Hong, Soon-Cheol	Hospital for Sick Children
Houle, Steeve	Warnex Medical Laboratories
Huguet, Jade	CRCHUM - Université de Montréal
Hutson, Janine	University of Toronto
Ito, Shinya	Hospital for Sick Children
Jackson, Chris	McGill University
Jacqz-Aigrain, Evelyne	Robert Debre Hospital, Paris
Jahedmotlagh, Zahra	University of Western Ontario
Jain, Sarthak	University of Alberta
Jamali, Fakhreddin	University of Alberta
Jia, Yamin	Jack Bell Research Center
Jia, Wei	University of North Carolina at Greensboro
Jimenez, Ricardo	University of British Columbia

Jizi, Khadije	Université de Montréal
Johnson, Chris	Able Translations
Jong, Will	Hospital for Sick Children
Julien, Catherine	Faculty of Pharmacy-Laval University and IUCPQ
Jurgens, Tannis	College of Pharmacy, Dalhousie University
Kahouli, Imen	McGill, Experimental Medicine
Katiforis, John	BioPharma Services
Kelly, Lauren	Schulich School of Medicine and Dentistry
Kennedy, Deborah	The Hospital for Sick Children
Khatri, Nirav	The M.S.University of Baroda, India
Kim, Richard	University of Western Ontario
Kim, Joon	ActLabs Ltd.
Knauer, Michael	University of Western Ontario
Knoppert, David	St Joseph's Hospital, London
Knutson, Jennifer	Afexa Life Sciences
Kondratev, Dennis	Maxxam Analytics
Konidas, Catherine	Algorithme Pharma
Konkin, David	National Research Council
Koren, Gideon	Hospital for Sick Children
Kotra, Lakshmi	University of Toronto
Kremer, Richard	McGill University
Kumar, Rav	GlaxoSmithKline
Kvann, Soriya	Univerisité de Montréal
Lafrenière Bessi, Valérie	Univerisité de Montréal
Lai, Chien-Tsai	Afexa Life Sciences
Lalone, Andre	SOGC
La, Hoa	Able Translations
Lachmansing, Sagar	Activation Laboratories
Lam, Jessica	Hospital for Sick Children
Lambert-Lanteigne, Patrick	University of Montreal
Le, Xuan Tuan	Materials Engineering, McGill University
Lebel, Marcel	CHUQ Research Centre
Leblond Chain, Jeanne	Université de Montréal
Lecanu, Laurent	McGill University
Leclair, Grégoire	Université de Montréal
Lee, Candace	Montreal Heart Institute
Legan, Robin	London Health Sciences Centre
Lemieux, Marc	Université du Quebec a Montreal
Leung, Yat Hei	CRCHUM - UdeM
LeVanseler, Kerri	NSF International
Levine, Mitchell	Centre for Evaluation of Medicines
Levy, Adrian	Dalhousie University
Li, Yatong	University of Toronto
Li, Xiaodong	Amgen Inc
Liang, Chen	Afexa Life Sciences
Ling, Lei	Afexa Life Sciences
Litalien, Catherine	Clinical Pharmacology Unit, CHU Ste-Justine
Liu, Rui	University of Ottawa
Loo, Tenneille	University of British Columbia
Loughrey, Helen	Theratechnologies Inc
Lu, Sharon	University of Western Ontario
Lu, Jennifer	CHUM/Montreal University
Lu, Wenjie	Indianna University
Lussier, Madeline	Afexa Life Sciences
Lutsiak, Christie	Afexa Life Sciences
Lutter, Lorelei	BioPharma Services Inc.
Lysechko, Tara	Afexa Life Sciences
Ma, Brian	Afexa Life Sciences
Ma, Dennis	University of Windsor
MacDonald, Patrick	University of Alberta
MacKenzie, Erin	Afexa Life Sciences
MacLeod, Stuart	University of British Columbia
Madadi, Parvaz	Hospital for Sick Children
Maliba, Ricardo	IC Axon
Mansell LeMay, Sara	University of Western Ontario
Mao, Lilly	Afexa Life Sciences
Marceau, François	CHUQ Reseach Center
Marelli, Benedetto	McGill University
Margolese, Howard	McGill University Health Centre
Maria, Salwa	Faculty of Dentistry, McGill University
Marinescu, Daniel	McGill University
Marleau, Sylvie	Université de Montréal
Martel, Sylvain	Ecole Polytechniquede Montreal
Massey, Thomas	Dept. Pharmacology & Toxicology, Queen
Mateescu, Mircea Alexandru	Université du Québec à Montréal
Mathieu, Sophie	Montreal Heart Institute
Matienzo, Javier	Watson Laboratories
Matlow, Jeremy	Hospital For Sick Children

Matok, Ilan	Hospital for Sick Children
Matsui, Doreen	University of Western Ontario
McCoy, Valerie	LV Lomas Ltd
McCullough, Nancy	Retired
McDarby, Michael	Fulton-Montgomery Community College
McGilveray, Iain	McGilveray Pharmacon Inc.
McNeil, Shelly	Dalhousie University
Mellal, Katia	Université de Montréal
Melong, Jonathan	Dalhousie College of Pharmacy
Michaud, Josee	University of Montreal
Michaud, Veronique	Indiana University
Michel, Deborah	University of Saskatchewan
Miller, Donald	University of Manitoba
Miller, Sandra	Afexa Life Sciences
Mohammed-Saeid, Waleed	University of Saskatchewan
Morgan, Alex	University of Western Ontario
Mulumba, Mukandila	Université de Montréal
Murch, Susan	University of British Columbia
Murray, Tamara	Afexa Life Sciences
Murty, Mano	Health Canada
Musallam, Lina	Université de Montréal
Nachar, Abir	Université de Montréal
Narine, Kelly	Afexa Life Sciences
Narwaley, Malyaj	University of Alberta
Natekar, Aniket	Hospital for Sick Children
Naud, Judith	University of Montreal
Nesbitt, Stephanie	Fulton-Montgomery Community College
Neufield, Katherine	Health Canada
Neuman, Manuela	MaRS Discovery District
Newa, Madhuri	University of Alberta
Ngovandan, Nicholas	L. V. Lomas
Nouri, Nada	Private sector
Nulman, Irena	Hospital for Sick Children
Ogilvie, Richard	University of Toronto
On, Ngoc	University of Manitoba
Ouellet, Jean	McGill University - MUHC/Shriners
Ouellette, Francis	Ontario Institute for Cancer Research
Ovadje, Pamela	University of Windsor
Ovakim, Daniel	University of Western Ontario
Pandey, Mitali	Vancouver Prostate Centre
Pandey, Siyaram	University of Windsor
Pang, Catherine	University of British Columbia
Parfrey, Patrick	Memorial University of Newfoundland and Labrador
Paris-Robidas, Sarah	Centre de recherche du CHUL
Parkinson, Fiona	University of Manitoba
Patel, Vishal	University of Western Ontario
Picard, Melanie	CRBM
Piquette-Miller, Micheline	University of Toronto
Poirier, Hugo	Boehringer Ingelheim (Canada) Ltd.
Pollak, P. Timothy	University of Calgary
Poon, Calvin	ISMP Canada
Poupart, Paul	Agilent Technologies
Prasad, Preethy	University of Toronto
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Provost, Marie	Clef des Champs
Quiniou, Christiane	Hopital Ste-Justine, Université de Montréal
Ramcharran, Ram	Sotax (Canada) Ltd.
Ramteke, Kuldeep	Dirugarh University, Assam
Rapinski, Michel	IRBV - University of Montreal
Rebry, Rachel	Nutrasource Diagnostics Inc.
Remirez, Diadelis	National Ctr for State Qualtiy Control of Drug
Renzi, Paolo	University of Montreal
Reynolds, James	Queen's University
Riddick, David	University of Toronto
Rieder, Michael	University of Western Ontario
Rigout, Nicole	L V Lomas
Rittenbach, Kay	Afexa Life Sciences
Robertson, Rebecca	NHPRS
Rogers, Kristian	LV Lomas
Royer, Mariana	Université Laval
Rui, Liu	University of Ottawa
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Saha, Shyamali	McGill University
Sanaee, Forugh	University of Alberta
Sanchez, Veronica	University of Western Ontario
Santilli, Kavita	Quality Compliance Labs
Saraiva, Elizabeth	World Courier

Sawynok, Jana	Dalhousie University
Sayani, Amyn	GlaxoSmithKline
Schwarz, Ute	Division of Clinical Pharmacology, UWO
Scorza, Fabien	CAMAG Scientific Inc
Searle, Gavin	Afexa Life Sciences
Seedoyal, Nahmratta	Afexa Life Sciences
Selke, Mike	Sheffield Bio-Science
Semple, Hugh	Alberta Innovates - Technology Futures
Seng Yue, Corinne	Université de Montréal
Shan, Jacqueline	Afexa Life Sciences
Shang, Nan	Universite de Montreal
Shao, Wei	McGill University
Sharma, Arya	University of Alberta
Shau, Hungyi	National Institutes of Health, NCCAM
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Sitar, Daniel	University of Manitoba
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Smith, Alysyn	Health Canada
Smith, Mary	Accucaps Industries Ltd.
Smith, Anne	Pharmaceutical Outcomes Programme, BC Children
Smith, Michael	Health Canada
Soldin, Offie	Georgetown University Medical Ctr
Steffen, Thomas	Orthopaedic Research Laboratory
Stern, Seth	Hospital for Sick Children
Sugiyama, Daisuke	The University of Western Ontario
Sun, Chao	University of Manitoba
Sun, Zhizhi	University of Manitoba
Sutherland, Sharla	Afexa Life Sciences
Sutherland, Sharla	Afexa Life Sciences
Sylvestre, Jean-Philippe	Département de génie physique
Ta, Chieu Anh	University of Ottawa
Tahiri, Houda	Université de Montréal
Takitani, Kimitaka	Osaka Medical College
Tamimi, Faleh	McGill University
Tanaka, Toshihiro	Hitachinaka General Hospital
Tanielian, Lory	Gattefosse Canada
Tardif, Jean-Claude	Montreal Heart Institute
Tartari Pereira, Priscilla	University of São Paulo
Teft, Wendy	The University of Western Ontario
Thibault, Marc	Ecole Polytechnique
Thibert, Roch	Corealis Pharma
Thomas, Dave	Brightwell
Tiedemann, Kerstin	McGill University
Tirunellai, Krishnan	Health Canada
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Tomlinson Guns, Emma	NHPRS
Touyz, Rhian	University of Ottawa
Tracy, Mark	Alnylam
Turgeon, Jacques	CRCHUM-Université de Montréal
Turi, Christina	University of British Columbia
Uludag, Hasan	University of Alberta
Ungar, Wendy	Hospital for Sick Children
Urquhart, Brad	University of Western Ontario
Usher, Lindsey	University of Saskatchewan
Utgikar, Rucha	University of Toronto
Vadas, Elizabeth	InSciTech Inc.
Vallee, Marie-Helene	PharmaNet Canada
van Blitterswijk, Clemens	University of Twente
vanderVaart, Sondra	The Hospital for Sick Children
Vázquez, Ana Maria	Center of Molecular Immunology
Velazquez, Carlos	University of Alberta
Velenosi, Thomas	University of Western Ontario
Viau, Martin	Université de Montréal
Villano, Jonathan	Cedarlane
Vohra, Sunita	University of Alberta
Walshe-Roussel, Brendan	University of Ottawa
Wang, Stan	Afexa Life Sciences
Wang, Meiqi	Afexa Life Sciences
Warner-Bell, Bree	Colorcon
Wasan, Kishor	University of British Columbia
Waye, Andrew	University of Ottawa
Wehner, Sue	Med-Script Associates Ltd
Winston, David	Herbalist & Alchemist Inc.
Winters, Conrad	Xenon Pharmaceuticals Ltd.
Wishart, David	University of Alberta
Wong, Ting	Afexa Life Sciences
Wong, Ian	University of British Columbia
Woolsey, Sarah	University of Western Ontario

Wu, Yuchin	University of Alberta
Xenocostas, Anargyros	LHSC-University of Western Ontario
Xiang, Fuli	University of Western Ontario
Xu, Jinke	McGill University
Yang, Guangdong	Lakehead University
Yazdani-Brojeni, Parvaneh	Hospital for Sick Children
Yeung, Pollen	Dalhousie University
Young, Robert	Simon Fraser University
Zelner, Irene	Hospital for Sick Children
Zeng, Jinqi	Health Canada
Zeyad, Alehaideb	Simon Fraser University
Zhang, Junzeng	National Research Council Canada
Zhang, Yu Ling	McGill University
Zhang, Xiaochu	University of Toronto
Zhou, Kangbin	University of British Columbia
Zouki, Christine	Boehringer Ingelheim (Canada) Ltd.

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