Modern Therapeutics 2012:

Advances in Physiology, Pharmacology, And Pharmaceutical Sciences

June 12-15, 2012
University of Toronto
Toronto, ON, Canada

A joint conference of:

Canadian Society for Pharmaceutical Sciences
Canadian Society for Pharmacology & Therapeutics
Canadian Physiological Society

Conference Management Committee:

CSPS: Laszlo Endrenyi, Barbara Scollick
CSPT: Kathy Gaebel, Fiona Parkinson, Cindy Woodland
CPS: Scott Heximer

Welcome to Toronto! This is our 15th Annual Symposium and this year we are pleased to collaborate with two scientific societies - the Canadian Society for Pharmacology and Therapeutics, and the Canadian Physiological Society - to bring you the program: Modern Therapeutics 2012: Advances in Physiology, Pharmacology, and Pharmaceutical Sciences.

The Canadian Society for Pharmaceutical Sciences (CSPS) is a non-profit organization established in 1996 to foster excellence in pharmaceutical research. CSPS membership includes scientists world-wide, who are involved in all aspects of pharmaceutical sciences with affiliations ranging from academia, industry to government. The electronic Journal of Pharmacy and Pharmaceutical Sciences is the official, international journal of CSPS. One of our major objectives is to build partnerships and develop a strong voice to encourage government, academia, and industry to advance pharmaceutical R&D innovation in Canada.

CSPS also offers a variety of events on timely topics, designed to engage and educate our members, both face-to-face and through webinars.

CSPS Board of Directors (from Nova Scotia to British Columbia, from government, industry and academia):

> Robert Young, Raimar Löbenberg, Laszlo Endrenyi, Lorelei Lutter, Neil Berger, Fakhreddin Jamali Christine Allen, Jake Barralet, Elisabeth Kovacs, Grégoire Leclair, Kishor Wasan, Pollen Yeung, Preethy Prasad, Frank Burczynski, Krishnan Tirunellai.

Scientific Program Chairs for 2012: Reina Bendayan and Murray Ducharme

Not only do we hope you find the conference sessions to be thought-provoking and valuable, we hope you spend time meeting old friends and making new ones. Enjoy Toronto!

Robert Young
President of CSPS (2012 – 2013)

CSPS 2012 Awards:

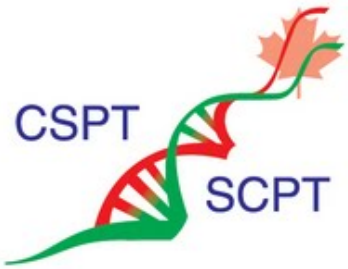
CSPS Award of Leadership in Canadian Pharmaceutical Sciences: Leslie Dan (Teva Canada, Toronto)

GlaxoSmithKline/CSPS Early Career Award: Ildiko Badea (University of Saskatchewan)

Gattefossé Canada/CSPS Lipid-Based Drug Delivery Award: Jagbir Singh (University of Saskatchewan)

GSK National Summer Student Research Program Awards: Christopher Chen (University of Alberta), Muffadal Shamshuddin (University of British Columbia), Kathryn Landry (Dalhousie University), Jordan Nash (University of Manitoba), Stephanie Hewitt (Memorial University of Newfoundland), Arwa El-Housseini (Université de Montréal), Heather Duerksen (University of Saskatchewan), Chonguk Allan Choi (University of Toronto).

Poster Awards (TBA): Cedarlane Award of Excellence; Antoine A. Noujaim Award of Excellence



Canadian Society of Pharmacology and Therapeutics

Welcome to Toronto! It is my pleasure to welcome you to the 2012 annual meeting of CSPT, which is being held jointly with the Canadian Society for Pharmaceutical Sciences and the Canadian Physiology Society. This year's conference is entitled "Modern Therapeutics 2012: Advances in Physiology, Pharmacology and Pharmaceutical Sciences." I am confident you will find it an exciting scientific program and an excellent opportunity to network with colleagues in related disciplines. I'd like to offer an extra warm welcome to students and other trainees. I hope you find the conference a valuable experience that enhances your training and your career development!

CSPT is a non-profit organization devoted to the promotion of research and education in pharmacology and therapeutics. Members of the Society include students, post-doctoral fellows, established researchers and clinician scientists from academia, government and industry. Our Society is run by volunteers together with our multi-talented Executive Director, Kathryn Gaebel. The current executive members are Fiona Parkinson, president; Richard Kim, past-president; Shinya Ito, vice-president; Cindy Woodland, treasurer; and Catherine Pang, chair of the Scientific Program Committee. The Society also has an active Awards Committee, chaired by Shinya Ito. Please bring forward to any of these individuals your suggestions for future meetings or your comments on other Society activities.

I would also like to thank the organizations that have provided financial support to our Society and to this meeting. These organizations are identified on the back page of this booklet.

For more information on CSPT activities please visit our website at http://www.pharmacologycanada.org/

Best wishes,

Fiona Parkinson
President, CSPT

CANADIAN PHYSIOLOGICAL SOCIETY
LA SOCIÉTÉ CANADIENNE de PHYSIOLOGIE

The Canadian Physiological Society is pleased to welcome you to this meeting jointly hosted by the Canadian Society for Pharmacology and Therapeutics (CSPT) and the Canadian Society for Pharmaceutical Sciences (CSPS), titled "Modern Therapeutics 2012: Advances in Physiology, Pharmacology and Pharmaceutical Sciences".

The Canadian Physiological Society exists to encourage research in the Physiological Sciences and to foster communication between members of the Canadian scientific community. It serves as a forum for the dissemination and discussion of scientific information of interest to researchers in Physiology and related Biological Sciences.

Our Annual meeting is being held June 12-15th at the University of Toronto.
We thank our Organizing Committee (CPS): Scott Heximer, Zhong-Ping Feng (co-chairs, University of Toronto); Doug Jones (Western University), Melanie Woodin (University of Toronto), and Deda Gillespie (McMaster University).

We are pleased to promote the Sarrazin Lecture, a tradition of the society since 1977. This lectureship was named in recognition of the "First Canadian Physiologist", Dr. Michel Sarrazin (1659-1734). This year we are delighted to welcome Dr. Duncan Stewart, CEO and Scientific Director of the Ottawa Hospital Research Institute. Dr. Stewart is a pioneering Canadian cardiovascular researcher, who is recognized for his many important discoveries in blood vessel biology as well as his dedication to translating these discoveries into benefits for patients and society. His presentation of the 2012 The Sarrazin Lecture is scheduled for Thursday, June 14th from 4:30-6 pm. We encourage all to attend.

We also are pleased to announce that this year's Stevenson Award is presented to Dr. Deda Gillespie, from the Department of Psychology, Neuroscience and Behaviour at McMaster University. Deda will present a lecture entitled: ***"Wired for Sound: establishing excitatory-inhibitory balance in auditory brainstem".*** We encourage attendance. Check the schedule.

The Canadian Physiological Society is a member of the International Union of Physiological Sciences (IUPS). We encourage members to plan on attending the 37th Congress of the International Union of Physiological Sciences scheduled for the International Convention Centre at Birmingham, UK, July 21-26, 2013.

The Executive of The Canadian Physiological Society welcome you to the meeting in Toronto and hope that you enjoy a stimulating conference.

Stephen Sims

President, Stephen Sims; Past President, Douglas Jones; Secretary, Melanie Woodin;
Treasurer, Catherine Chan
Councillors: Elizabeth Cowley, Michael Jonz, Gerald Zamponi, Lingyun Wu, William Cupples, Deda Gillespie
Nominating Committee: Doug Jones, Scott Heximer, Katalin Toth, Bruce Mathieson

Conference Program

Tuesday June 12	
08:00-16:30	**PRE-CONFERENCE WORKSHOP**: "**Drug Product Development: A QbD Approach**" Presented by CSPS (Registration fees extra, not included in conference registration, lunch included) (Pharmacy Building Room 850)
11:30-12:00	**Registration for Trainee Workshop and light lunch** (Med Sci Bldg MacLeod Auditorium Lobby)
12:00-17:00	**TRAINEE WORKSHOP: TECHNICAL SKILLS OF SUCCESSFUL SCIENTISTS** (Med Sci Bldg - MacLeod Auditorium) Co-chairs: Fiona Parkinson and Donald Miller 12:00-12:10 Welcome **12:10-12:40** **Small Molecule Binding can Interfere with Helix-helix Interactions and Modulate Amyloid Generation** Gerhard Multhaup (McGill University) **12:45-13:15** **AFM-Based Force Sensing on Individual Cells; Applications in Pharmacology** Michel Grandbois (Université de Sherbrooke) **13:20-13:50** **Drug Safety: Completing Clinical Trials with Real World Experience** David Juurlink (ICES) **13:55-14:25** **Centre for Clinical Investigation & Therapeutics: Enabling Technologies for Patient Oriented Research in the 21st Century** Richard Kim (University of Western Ontario) **14:30-14:50 Break** **14:55-15:20** **Multidisciplinary Approaches Towards Understanding Ion Channel Function in the Nervous System** Gerald Zamponi (University of Calgary) **15:30-16:00** **Applications of Computer Aided Drug Design to High Throughput Screening and Rational Discovery Strategies** Donald Weaver (Dalhousie University) **16:05-16:35** **Model Systems for Biophotonic Imaging of Brain Responses to Injury and Therapies** Jasna Kriz (Laval University)
17:00-20:00	CSPT Executive Board Meeting (Bumpkins Restaurant)
17:15-19:00	Trainee Social - O'Grady's Pub, College Street
18:30	CSPS Board of Directors Meeting (Delta Chelsea Hotel - Whistler Boardroom)

Wednesday June 13	
07:00-	Registration opens, Coffee & Muffins (Med Sci Bldg MacLeod Auditorium Lobby**)**
08:00-08:30	Welcome
08:30-09:30	**Plenary 1: PHARMACOLOGICAL NEUROPHYSIOLOGY (**Med Sci Bldg - MacLeod Auditorium**)** Chair: Fiona Parkinson **All Roads to Psychosis Pass Through Dopamine Supersensitivity and Elevated D2High States** Philip Seeman (University of Toronto)
09:30-10:00	NUTRITION BREAK AND VIEWING OF POSTERS AND EXHIBITS
10:00-12:15	**Plenary 2: CLINICAL PRACTICE GUIDELINES FOR PHARMACOGENETIC TESTING: THE FIRST SIX DRUGS (**Med Sci Bldg - MacLeod Auditorium**)** Chairs: Shinya Ito and Bruce Carleton **Overview of Workshop** Shinya Ito (Hospital for Sick Children) **CSPT/CSPS State of the Art Lecture; MapQuest for Pharmacogenetic Tests: How to Arrive at Improved Clinical Outcomes** Larry Lesko (University of Florida) **CPG Development Process** Bruce Carleton (University of British Columbia) **Guideline for Pharmacogenetic Testing: Carbamazepine** Ursula Amstutz (University of British Columbia) **Recommendations: Codeine** Parvaz Madadi (Hospital for Sick Children) **Recommendations: Cisplatin** Colin Ross (University of British Columbia) **Recommendations: Tamoxifen** Ricardo Jimenez (University of British Columbia) **Recommendations: Anthracyclines** Colin Ross (University of British Columbia) **Recommendations: Warfarin** Kaitlyn Shaw (University of British Columbia) **Next Steps, Distribution of CPG Materials** Bruce Carleton (University of British Columbia)
12:30-13:30	LUNCH (MSB-Cafeteria Room beside Starbucks) AND VIEWING OF POSTERS AND EXHIBITS
13:00-13:30	**Corporate Symposia** (Med Sci Bldg - Cafeteria Room beside Starbucks)

13:30-16:00	**ALZHEIMER'S DISEASE** (Med Sci Bldg MacLeod Auditorium) Chairs: Claudio Cuello, Zhong-Ping Feng **The Role of AMPAR Trafficking in Memory Deficits Observed in Mouse Models AD** Sheena Josselyn (University of Toronto) **Molecular Mechanisms Regulating the Abeta Amyloid Production** Weihong Song (UBC) **Caspases as Causative Agents in Alzheimer's Disease** Andrea Leblanc (McGill University) **Neurotrophin Regulation and Cognitive Impairment in Alzheimer's Disease** Margaret Fahnestock (McMaster University) **Early Inflammation and NGF Deregulation in Alzheimer's and MCI** Claudio Cuello (McGill University)	**CANCER STEM CELLS: NEW POTENTIAL CLINICAL TARGETS** (Med Sci Bldg Rm 3153) ***Sponsored by FMC BioPolymer*** Chair: Feridoun Karimi-Busheri **Basic and Translational Biology of Cancer Stem Cells** William Matsui (Johns Hopkins University) **Targeting Cancer Stem Cell-Specific Molecular Pathways** Markus Frank (Harvard Medical School) **Targeting Cancer Stem Cells: Promises and Challenges Ahead** Feridoun Karimi-Busheri (NovaRx & University of Alberta) **Panel Discussion**	**THE NEW CANADIAN BE GUIDELINES IN THE MIDST OF CURRENT CHALLENGES FROM A REGULATORY AND INDUSTRY POINT OF VIEW** (Pharmacy Bldg Rm 850) ***Sponsored by BASF*** Chairs: Isadore Kanfer and Raimar Löbenberg **The New Canadian Bioequivalence Guidelines** Eric Ormsby (Health Canada) **Bioequivalence Review Challenges in a Changing Regulatory Environment** Jaigi Mathai (Health Canada) **Challenging Issues on the Demonstration of Bioequivalence** Yu Chung Tsang (Apotex) **Panel Discussion**
16:00-18:00	**Poster Session** (Med Sci Bldg Stone Lobby)		
16:00-16:30	**Senior Investigator Award Lecture** (Med Sci Bldg MacLeod Auditorium) **From Drugs to Patients: Personalizing Care, not Medicines** Bruce Carleton (University of British Columbia)		
18:00-20:00	Joint opening reception (Faculty Club)		

Thursday June 14

07:00 Registration opens, Coffee & Muffins (Med Sci Bldg MacLeod Auditorium Lobby)

08:00-10:00

FOOD INTAKE AND REWARD
(Med Sci Bldg MacLeod Auditorium)

Chair: Stephanie Borgland

Effects of Insulin on VTA Dopamine Neurons
Stephanie Borgland (University of British Columbia)

Negative Emotional States and Neurobehavioral Adaptations in Brain Reward Circuitry Induced by Chronic High-fat Feeding
Stephanie Fulton (Université de Montréal)

Ghrelin Modulation of Dopamine Neurotransmission
Alfonso Abizaid, (Carleton University)

Co-sensitivity to the Incentive Properties of Palatable Food and Cocaine in Rats; Implications for Comorbid Addictions
Francesco Leri (University of Guelph)

A Psychogenetic Study of Food Addiction in Adult Men and Women
Caroline Davis (York University)

08:00-09:00

AWARD LECTURES
(Med Sci Bldg Rm 3153)

Chair: Dion Brocks

GlaxoSmithKline/CSPS Early Career Award Lecture:

Non-invasive Drug Delivery – Rational Design and Characterization of Nanoparticulate Delivery Systems

Ildiko Badea (University of Saskatchewan)

CSPS Award of Leadership in Canadian Pharmaceutical Sciences

Leslie Dan (Teva)

09:00-10:00
(Med Sci Bldg Rm 3153)

Chair: Reina Bendayan

Advances in Research of Drug Transporters and Metabolic Enzymes: Implications in Drug Development
Yuichi Sugiyama (University of Tokyo)

CPS ORAL PRESENTATIONS
(FitzGerald Bldg Rm 103)

Hyperpolarizing GABAergic Transmission Requires the KCC2 C-terminal ISO Domain
Brooke Acton (University of Toronto)

Importance of the Mevalonate Pathway in the Development and Survival of Purkinje Cells
Andrew Barszczyk (University of Toronto)

Glutamate and GABA/Glycine Co-released in the Immature Inhibitory MNTB-LSO Circuit Show Differential Dependence on Calcium
Javier Alamilla (McMaster University)

Hydrogen Bonds are Critical for the Docking and Formation of Functional CX26/CX32 Gap Junction Channels
Donglin Bai (University of Western Ontario)

Intracellular Trafficking of Regulator of G-protein Signaling 4 (RGS4) Requires Palmitoylation and RAB Family Function
Bastin Guillaume (University of Toronto)

10:00-10:30 NUTRITION BREAK AND VIEWING OF POSTERS AND EXHIBITS

10:30-12:30	**CHALLENGING REAL PATIENT PHARMACOLOGY/ TOXICOLOGY CASES PRESENTING AT CANADIAN HOSPITALS** (Med Sci Bldg MacLeod Auditorium) Chair: George Dresser	**NANOTECHNOLOGY IN CANCER THERAPEUTICS: FROM DETECTION TO ERADICATION** (Med Sci Bldg Rm 3153) ***Sponsored by Purdue Pharma*** Chairs: Shirley Wu and Marianna Foldvari **Nanoparticle Delivery of siRNA & Chemotherapy to Tumors** Leaf Huang (University of N. Carolina Chapel Hill) **Drug Delivery with Mitochondria-penetrating Peptides** Shana Kelley (University of Toronto) **Lipid Nanoparticle Formulations of siRNA for Gene Silencing in the Liver** Pieter Cullis (UBC) **Nanoscale Delivery of Drug Combinations for Overcoming Multidrug Resistance in Cancer** Shirley Wu (University of Toronto)	**NEUROSCIENCE SYMPOSIUM** (FitzGerald Bldg Rm 103) Chairs: Melanie Woodin and Zhong-Ping Feng **Presynaptic Vesicle Recycling Mechanisms and Information Transfer** Katalin Toth (Université Laval) **New Molecules Enhancing Axonal Outgrowth and Regeneration** Zhong-Ping Feng (University of Toronto) **Electrical Microstimulation: Modifying Neural Circuits that are Linked to Cognitive Function** Erik Cook (McGill University) **Treatment of Stroke using PSD-95 Inhibitors** Michael Tymianski (Toronto Western Research Institute)
12:30-14:00	**CSPT**: Med Sci Bldg MacLeod Auditorium - LUNCH and Annual General Meeting **CSPS**: Med Sci Bldg Rm 3153 - LUNCH, AGM and CSPS Award Presentations **CPS**: Med Sci Bldg Rm 2173 - LUNCH and Annual General Meeting VIEWING OF POSTERS AND EXHIBITS		

14:00-16:30	**14:00-16:00** **CSPT TRAINEE ORAL PRESENTATIONS** (Med Sci Bldg MacLeod Auditorium) **HLA-B*1502 and HLA-A*3101 as Genetic Markers for Carbamazepine-induced Hypersensitivity Reactions in Children** Ursula Amstutz (University of British Columbia) **Effect of Human Equlibrative Nucleoside Transporter 1 and Ecto-5'nucleotidase (eN) in Adenosine Formation by Astrocytes under Ischemic Conditions** Stephanie Chu (University of Manitoba) **The Effect of N-acetylcysteine on the Antitumour Efficacy of Ifosfamide in a Mouse Xenograft Model** Lauren Hanly (University of Western Ontario) **Investigation of the Cytotoxic Effects of Novel Jadomycins in Drug-sensitive and Drug-resistant Breast Cancer Cells** Mark Issa (Dalhousie University) **Ethyl Glucuronide Crosses the Human Placenta and Represents Maternal and Fetal Exposure to Alcohol** Jeremy Matlow (Hospital for Sick Children) **Embryonic catalase protection against ethanol embryopathies in acatalasemic and human catalase-expressing mice in embryo culture** Lutfiya Miller (University of Toronto) **Lysophosphatidicacid (LPA)-**	**ADVANCES IN LIPID-BASED NANOPARTICULATE SYSTEMS FOR DRUG AND VACCINE DELIVERY** (Med Sci Bldg Rm 3153) ***Sponsored by Tilak Technologies*** Chairs: Kishor Wasan and Panayiotis Constantinides **Parenteral and Oral Lipid Nanodispersions for Small Molecule and Macromolecule Delivery: Biopharmaceutical Considerations and Case Studies** Panayiotis Constantinides (Biopharmaceutical & Drug Delivery Consulting) **Multi-Compartmental Lipid Delivery Systems for Cancer Vaccination** Mansoor Amiji (Northeastern University) **Transcutaneous DNA Immunization: From Nanoparticles to Hair Follicles and Back to Nanoparticles** Zhengrong Cui (University of Texas at Austin) **BCS Class IV Compound Case Study: Development and Evaluation of a Novel Oral Amphotericin B Formulation for the Treatment of Systemic Fungal Infections and Drug-Resistant Visceral Leishmaniasis (VL)** Kishor Wasan (UBC)	**CONDUCTING BE STUDIES AND OTHER CLINICAL TRIALS IN CANADA AND FOREIGN COUNTRIES** (Pharmacy Bldg Rm 850) Chairs: Lorelei Lutter and Fethi Trabelsi **Canadian Sponsor Perspectives: Challenges and Opportunities in Outsourcing Bioequivalence Studies** Manon Belisle (Teva Canada) **BE Study Conduct in Canada, Pakistan, Poland and Egypt: A Canadian CRO Perspective** Lorelei Lutter (BioPharma Services) and Levon Yeghikyan (BioPharma Metrics, Pakistan) **Running Clinical Trials in India: History, Current Environment, and Prospects for the Future** Alfred Elvin (Anatase Biopharmaceutic Consulting, USA) **Outsourcing or In-house of Conduct of BE Studies in Canada, US and Rest of the World - Create Competitive Advantage** Hari Sankar (Watson Pharmaceuticals, India) **Recommendations for Improving Quality of Bioequivalence Data Submitted to ANDAs in the US** Barbara Davit (US FDA)

	induced Enhancement of Blood-brain Barrier (BBB) Permeability as a Potential Method for Enhancing Drug Delivery to the Brain Ngoc On (University of Manitoba) **Ligand-dependent and Receptor-selective Effect of Non-nucleoside Reverse Transcriptase Inhibitors on the Activity of Human Pregnane x Receptor and Constitutive Androstane Receptor** Devinder Sharma (University of British Columbia) **16:00 - 16:30** **CSPT Distinguished Service and Education Award Lecture** **The Challenges of Pharmacology in the New Millennium** Patrick du Souich (Université du Montreal)		
16:30-18:00	**Poster Session** (Med Sci Bldg Stone Lobby)		
16:30-18:30	**Canadian Physiologic Society Distinguished Lectureship Series** (Med Sci Bldg MacLeod Auditorium) Chair: Stephen Sims **JAF Stevenson Visiting Professor Lecture:** **Wired for Sound: establishing excitatory-inhibitory balance in auditory brainstem** Deda Gillespie (McMaster University) **Sarrazin Award Lecture:** **Towards Modern Therapeutics for Pulmonary Vascular Disease: Inspiration from New Mechanistic Insights** Duncan Stewart (Ottawa Heart Institute)		
18:00-19:00	**Mixer (cash bar)** (Delta Chelsea Hotel - Mountbatten Salon)		
19:00	**CONFERENCE DINNER** (Delta Chelsea Hotel - Mountbatten Salon)		

Friday June 15			
08:00	Registration opens. Coffee & Muffins (Med Sci Bldg MacLeod Auditorium Lobby & Pharmacy Bldg Room 250**)**		
08:15-08:30	CSPT Award Presentations (Med Sci Bldg MacLeod Auditorium)		
8:30-10:30	**CARDIOVASCULAR DISEASE AND GLOBAL HEALTH** (Med Sci Bldg MacLeod Auditorium) ***Sponsored by NRC Research Press*** Chairs: Catherine Pang, Scott Heximer **Diabetes Therapeutics and Cardiovascular Disease** Daniel Drucker (Mount Sinai Hospital) **New Concepts in the Physiology of Sudden Cardiac Death in the Young** Michael Gollob (University Ottawa Heart Institute) **Genetic Predisposition for CAD** Robert Roberts (University of Ottawa Heart Institute) **Cardiometabolic Risk: Role of Genetics** Robert Hegele (University of Western Ontario)	**OBSTACLES IN DRUG ABSORPTION WITH EMPHASIS ON DISEASE AND PATHOPHYSIOLOGICAL CONDITIONS** (Pharmacy Bldg Rm 250) Chairs: Fakhreddin Jamali and Nikoletta Fotaki **Absorption Profile may be Different Between Healthy Volunteers and Patients: Obstacles in Drug Development; Do Animal Models Help?** Fakhreddin Jamali (University of Alberta) **Absorption Profile in Special Populations: Effect of Age** Nikoletta Fotaki (University of Bath, UK) **Bioavailability Profile as Predicted by Simulation Approaches** Raimar Löbenberg (University of Alberta) **Panel Discussion**	
10:30-11:00	NUTRITION BREAK		

11:00-13:00	**NEW INSIGHTS IN NEPHROTOXICITY IN CHILDREN** (Med Sci Bldg MacLeod Auditorium) Chair: Michael Rieder **Drug-Induced Renal Injury: Burden of Illness and Implications for Drug Development and Regulation** Marissa Battistella (University Health Network) **Drug-Induced Renal Injury in Special Populations: Neonatology and Oncology** Michael Rieder (University of Western Ontario) **New Mechanisms of Drug-Induced Renal Injury** Brad Urquhart (University of Western Ontario) **When the Kidney Produces its own Poisons** Gideon Koren (The Hospital for Sick Children)	**OPTIMIZING DRUG DEVELOPMENT USING BIOMARKERS AND BIOWAIVERS** (Pharmacy Building Room 250) Chairs: Pollen Yeung and Yu Chung Tsang **Approaches to Biomarkers and their use in Drug Development : A Regulatory Perspective** Agnes Klein (Health Canada) **ATP Metabolism as Biomarker Target for Drug Development** Pollen Yeung (Dalhousie University) **The Role of Biowaivers in Drug Development** Raimar Löbenberg (University of Alberta) **Modified Release Formulations: Waiver for Bio-studies for Different Dosage Strengths of a Drug Product Manufactured for Global Market** Jasmina Novakovic (Apotex) **11:00 - 13:00** **CSPS TRAINEE ORAL PRESENTATIONS** (Pharmacy Building Room 850) Chairs: Grégoire Leclair and Preethy Prasad (Speakers TBA)	**CPS ORAL PRESENTATIONS** (Fitzgerald Bldg Rm 103) **Hydrogen Sulfide *S*-sulfhydrates Pyruvate Carboxylase and Stimulates Gluconeogenesis** Guangdong Yang (Lakehead University) **Cardiac Specific Over-Expression of Membrane-Associated Human Stem Cell Factor Promotes Epicardial Activation Post Myocardial Infarction** Fu-Li Xiang (University of Western Ontario) **Atrial Fibrillation is Associated with Increased K_{ATP} Channel Function in RGS4-deficient Mice** Alexandra Mighiu (University of Toronto) **Localized Elevation of Cytosolic Free Calcium is Required for Uropod Retraction and Osteoclast Migration** Benjamin Wheal (University of Western Ontario) **Genome-wide RNAI Screening Identifies Drosophila Bestrophin 1 as a Swell-activated Chloride Channel** Stephanie Stotz (Harvard Medical School)
13:00-14:30	**Trainee Mentoring Luncheon** (O'Grady's Pub, College Street)		
13:00-14:00	Chairs of Pharmacology Departments Meeting (lunch provided) Dean's Conference (Med Sci Bldg Rm 2317)		

Table of Contents

CSPS AWARD LECTURES

CPS ORAL PRESENTATIONS

CHALLENGING REAL PATIENT PHARMACOLOGY/TOXICOLOGY CASES PRESENTING AT CANADIAN HOSPITALS

[Abstracts and bios not available]

NANOTECHNOLOGY IN CANCER THERAPEUTICS: FROM DETECTION TO ERADICATION

NEUROSCIENCE SYMPOSIUM

CSPT TRAINEE ORAL PRESENTATIONS

CSPT DISTINGUISHED SERVICE AND EDUCATION AWARD LECTURE

ADVANCES IN LIPID-BASED NANOPARTICULATE SYSTEMS FOR DRUG AND VACCINE DELIVERY

POSTER PRESENTATIONS DAY 1
WEDNESDAY, JUNE 13, 2012

CSPS POSTERS - DAY 1

Biomedical Sciences

Pharmaceutical & Analytical Chemistry

Pharmacokinetics & Pharmacodynamics

CSPT POSTERS - DAY 1

CSPT Trainee Poster Presentation Competition Abstracts

CIHR-DSEN Trainee Poster Presentation Award Abstracts

CSPT Poster Presentation Abstracts

POSTER PRESENTATIONS DAY 2
THURSDAY, JUNE 14, 2012

CSPS POSTERS - DAY 2

Biomedical Sciences

Clinical Sciences & Pharmacy Practice

Pharmacokinetics & Pharmacodynamics

Pharmaceutical & Analytical Chemistry

Drug Delivery & Pharmaceutical Technology

CSPT POSTERS - DAY 2

Speaker Abstracts

Tuesday, June 12, 2012

Trainee Workshop:

Technical Skills of Successful Scientists

Small Molecule Binding can Interfere with Helix-helix Interactions and Modulate Amyloid Generation

Gerhard Multhaup, McGill University, Montreal, Canada.

The key pathological agent of Alzheimer's disease (AD) is the amyloid-β (Aβ) peptide derived from a much larger protein, the amyloid precursor protein (APP).

Recently, we reported that the transmembrane sequence (TMS) of APP homodimerizes via the GxxxG interaction motif. Dimerization has a substantial impact on the specific isoform of Aβ produced. Engineered GxxxG mutants gradually attenuating the dimerization strength of the APP-TMS specifically decrease the production of the aggregation-prone and causative Aβ42 in favor of shorter Aβ species, e.g. Aβ38. Interestingly, the same beneficial effect on amyloid production was found for small compounds including sulindac sulfide and other non-steroidal anti-inflammatory drugs (NSAIDs).

We examined direct molecular interactions of sulindac sulfide and related compounds by SPR experiments and NMR spectroscopy. Sulindac sulfide and derived Aβ42-lowering compounds directly bind to the Aβ sequence and the alternating glycine residues of the GxxxG motif within the APP-TMS form an ideal contact site for those compounds as revealed by molecular modelling. To analyze their effect on APP-TMS dimerization stability in living cells we used a bacterial reporter gene-based dimerization assay (ToxR assay). Remarkably, we found that the helix-helix interaction of the APP-TMS is attenuated in a concentration-dependent manner by sulindac sulfide or other sulindac-related compounds.

Our data strongly suggest that small compounds as specific binding partners of the APP-TMS lower the production of Aβ42 by directly interfering with its dimerization. Taken together, we have identified a molecular link between APP-TMS dimerization and Aβ42 generation which classifies the APP-TMS as a high potential drug target.

Gerhard Malthaup
Dr. Multhaup received his PhD from the Institute of Genetics, University of Cologne. He was the Head of the Dept. of Biochemistry, Freie Universität Berlin before coming to McGill University. Dr. Multhaup's research interests are aimed at understanding the molecular, cellular and systemic mechanisms of degenerative nervous system disorders to yield knowledge relevant for the understanding, prevention, and treatment also of other acute and chronic neurological and psychiatric disorders. Research activities have a focus on basic mechanisms of nervous system function, and will further aim at analyzing critical cascades of neuronal toxicity and at unraveling endogenous neuroprotective mechanisms which may provide a basis for selective therapeutic interventions. Once neuronal toxicity has occurred, the brain utilizes principles of plasticity mechanisms in an attempt to recover function. Therefore, understanding these principles could lead to novel treatment strategies.

AFM-Based Force Sensing on Individual Cells; Applications in Pharmacology

Michel Grandbois, Canada Research Chair in Nanopharmacology and Atomic Force Microscopy; Dept. of Pharmacology, Université de Sherbrooke, Québec Canada

Activation of cell membrane receptor generally involves intracellular signaling cascades and molecular events susceptible to influence the overall

mechanical state of several types of cell, which in turn is expected to impact on several physiological functions. The Atomic Force Microscope, an important tool from the cell biophysics toolbox, can be applied to individual cells, to quantify and characterize the mechanical and morphological activity associated with the activation of G-protein coupled receptors (GPCR). By combining real-time force experiments with time-lapse sequences of phase contrast and confocal micrographs of cells transfected with GFP-tagged proteins, we show that receptor stimulation produces actin-dependent contractile responses with amplitude such as they are not easily detectable using usual optical techniques. By modulating the activity and/or expression levels of elements down stream of the receptor and acting as regulators of the acto-myosin dependent contractile activity we can delineate their contributions in the cell mechanical state. Thus, real-time force monitoring in single cells provides a label-free readout of GPCR-dependent molecular processes responsible for contractile activity and mechanical phenotype remodelling.

Michel Grandbois
Dr. Grandbois received his PhD in 1997 from the Université du Québec à Trois-Rivières and completed a Postdoc, Nanoscience, LMU Munich, Germany.

Before coming to the Université de Sherbrooke he was an assistant professor in the Physics Dept., University of Missouri-Columbia. He holds a Canada Research Chair and received the Natural Sciences and Engineering Research Council of Canada Postdoc fellowship.

Drug Safety: Completing Clinical Trials with Real World Experience

David Juurlink, Associate Professor of Medicine, Pediatrics, and Health Policy, Management, and Evaluation, Attending Physician, Division of General Internal Medicine, Head, Division of Clinical Pharmacology and Toxicology, Sunnybrook Health Sciences Centre, Scientist, Institute for Clinical Evaluative Sciences

Medications are generally brought to market because clinical trials demonstrate that they can effect some good. However, a drug's adverse effect profile is incomplete at the time of licensing, and its risk/benefit profile in clinical practice often differs from that seen in premarket studies. This lecture addresses the reasons for that, and using recent examples illustrates how thoughtful use of large healthcare databases can shed light on a drug's safety profile once it enters widespread clinical use.

David Juurlink
Dr. Juurlink is a General Internist at Sunnybrook Health Sciences Centre and the Director of the Division of Clinical Pharmacology at the University of Toronto. He is also a Medical Toxicologist at the Ontario Poison Information Centre and a Scientist at the Institute for Clinical Evaluative Sciences (ICES). He received degrees in Pharmacy and Medicine from Dalhousie University and completed postgraduate training in Internal Medicine, Clinical Pharmacology, and Medical Toxicology along with a PhD in Clinical Epidemiology, all at the University of Toronto. In addition to providing care to patients on the General Internal Medicine service, Dr. Juurlink directs the Sunnybrook Clinical Pharmacology and Toxicology consult service and elective rotation for senior medical residents, one of only a few such programs in Canada. He maintains an active research program in pharmaco-epidemiology, with a particular focus on drug safety and the clinical consequences of drug interactions. In addition to his research, clinical and teaching responsibilities, David serves on the Ontario Committee to Evaluate Drugs (CED), the Board of Trustees of the American Academy of Clinical Toxicology and the Royal College of Physicians and Surgeons Subspecialty Committee in Clinical Pharmacology.

Centre for Clinical Investigation & Therapeutics: Enabling Technologies for Patient Oriented Research in the 21st Century

Richard Kim, Professor of Medicine and Physiology & Pharmacology, and Oncology; Chair, Division of Clinical Pharmacology; Director, Centre for Clinical & Therapeutics; Department of Medicine, The University of Western Ontario

The goal of my presentation during this workshop is to highlight several key enabling technologies that has helped me to better understand the molecular determinants of intersubject variation in drug responsiveness. Using my career as an example, I will outline my first foray into the world of molecular biology in the early 1990s, just out of

residency training, with no formal training in molecular biology, but determined to learn and adopt heterologous gene expression strategies, which ultimately led to the successful cloning and expression of an array of drug transporters. I will then discuss my efforts at exploration and adoption of key genotyping technologies that were essential to large scale clinical pharmacogenomic studies in healthy subjects as well as in patients. I will also discuss advancements in drug level analysis technologies such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) to drug metabolism, transport, and pharmacogenomics studies which are often conducted as a part of clinical investigation, followed by a brief description of our newly built Centre for Clinical Investigation & Therapeutics (CCIT) for conducting clinical research which utilize such key technologies noted above, as our next logical and exciting step into Patient Oriented Research.

Richard B. Kim

Dr. Kim received his medical degree from the University of Saskatchewan in 1987. After completing an internship and residency training in Internal Medicine, he went on to carry out postdoctoral fellowship training in Clinical Pharmacology at Vanderbilt University. And then remained at Vanderbilt University in the Division of Clinical Pharmacology as a faculty member where he rose to the rank of Professor. In July of 2006, Dr. Kim and a number of his colleagues and post-doctoral fellows from his group moved from Vanderbilt University to the University of Western Ontario. He is currently Professor and Chair of the Division of Clinical Pharmacology in the Department of Medicine at the University of Western Ontario. He is leading a program of excellence in the field of Drug Transporters and Metabolizing Enzymes of relevance to Personalized Medicine.

Multidisciplinary Approaches towards Understanding Ion Channel Function in the Nervous System

Gerald Zamponi, Head, Department of Physiology and Pharmacology, University of Calgary

Voltage gated sodium calcium channels are key mediators of signalling in electrically excitable cells. They are pharmacological targets in a number of conditions such as pain and epilepsy, and mutations in sodium and calcium channel genes are associated with a wide range of pathologies ranging from Timothy syndrome to migraine. Our laboratory has studied these channels at the cellular, molecular and whole animal level. In my presentation, I will give an overview about some of the technical approaches that we have used to study these important membrane proteins, and how these approaches, and requirements for publication of ion channel work in high profile journals, have changed over the past two decades. I will also touch on aspects of drug discovery research outside of academia.

Gerald Zamponi

Dr. Gerald Zamponi is an internationally recognized expert on the biophysics, molecular biology, modulation, and pharmacology of ion channels and their role in nervous system function. His research focuses on exploring how these channels contribute to neurological disorders such as chronic pain and Alzheimer's disease. His goal is to develop strategies to regulate ion channel function for therapeutic intervention.

Dr. Zamponi's scientific work has garnered dozens of national and international awards. He has also authored more than 175 papers in top tier scientific journals. In addition, his research on ion channel therapeutics has resulted in numerous patents. Dr. Zamponi was appointed Head of the Department of Physiology and Pharmacology in 2008. In addition to being an AHFMR Scientist, he is the Canada Research Chair in Molecular Neurobiology and a Fellow of the Royal Society of Canada. Dr. Zamponi previously served as Research Director at the Hotchkiss Brain Institute and was a leader of the Epilepsy research program.

Applications of Computer Aided Drug Design to High Throughput Screening and Rational Discovery Strategies

Donald Weaver, Professor and Canada Research Chair in Clinical Neuroscience**,** Dalhousie University

This workshop presentation will give an overview of computer-aided drug design. Drawing from examples, based on epilepsy, dementia and infectious disease, methods and strategies for identifying and optimizing new chemical entities as

putative therapeutics will be discussed. First, methods for identifying an initial lead compound will be considered. In particular, three methods will be presented: rational drug design, in silico high throughput screening (HTPS) and analogues of endogenous compounds. Next, techniques for making the initial hit more drug-like will be included; Lipinski's rules and the role of algorithms for predicting brain penetration will be discussed. Finally, methods for compound optimization, including quantitative structure activity relationship (QSAR) calculations, will be reviewed. In addition, a very brief "high level overview" of the computational methods (e.g. empirical force field calculations) will be provided.

Donald Weaver
Donald Weaver is Canada Research Chair, T1, and Professor in the Departments of Medicine (Neurology), Chemistry and Biomedical Engineering at Dalhousie University. He is a practising clinical neurologist with a PhD in medicinal chemistry. He has published widely in the areas of drug design and computer-aided drug discovery. He has co-founded eight start-up biotech companies and holds more than 70 patents. He has received a number of awards for his drug design research including the Prix Galien and the Jonas Salk Award.

Model Systems for Biophotonic Imaging of Brain Responses to Injury and Therapies

Jasna Kriz, Department of Anatomy and Physiology, Laval University, Centre de Recherche du Centre Hospitalier de l'Université Laval

Recently, imaging strategies employing different reporter molecules have been developed to study biological processes as they occur in living animals or as they appear in real time in cell assays. These new technologies are based on sources of light emitted from fluorescent proteins such as GFP or luminescent enzymes (firefly luciferase - Fluc). Following the addition of appropriate substrate (luciferin), luciferase catalyses the cleavage of the substrate luciferin in presence of oxygen and ATP, resulting in the emission of light with broad spectral emission that peaks at 560 nm with substantial fraction of light above 600 nm making it suitable for in vivo imaging. The photons emitted by Fluc reporter activity pass the host tissue and are detectable at the surface with sensitive photo detectors based on a CCD camera. Using this approach in our laboratory we recently generated and validated in several new transgenic mouse models of bioluminescence and fluorescence for live imaging of processes associated with CNS injury and repair including inflammation/innate immune response, neuronal stress damage/recovery and neurogenesis. These mice represent unique tools for understanding brain responses to acute and chronic injuries and in vivo disease pathology. Furthermore, our recent studies strongly suggest that the biophotonic/bioluminescence signals imaged from the brains of live animals can be used as valid biomarkers to screen for novel biocompatible molecules and/or to visualize distinct pathological events and/or therapeutic efficacy.

Jasna Kriz
Dr. Jasna Kriz obtained her MD and PhD in Biomedicine & Health from University of Rijeka, Croatia followed by postdocoral training at the Centre for Research in Neuroscience of McGill University, Montreal, Canada (1999-2003). 2004 she was recruited at Laval University, Quebec City, Canada where she presently holds a title of Associate Professor at the Department of Psychiatry and Neuroscience, Faculty of Medicine Laval University. At 2006 she received R&D/HRF/CIHR (Canadian Institutes for Health Research) Career Award (Project entitled: Brain inflammatory response as a therapeutic target in cerebral ischemia) while presently she is recipient of the FRSQ Senior Scholarship Award. Her studies, using a transgenic model for selective ablation of proliferating microglial cells provided one of the first *in vivo* evidence of the neuroprotective potential of microglial cells in brain ischemia. Moreover to study inflammation and brain recovery from living animals and in real time she developed series of novel biophotonic (bioluminescence and fluorescence) transgenic models for *in vivo* analysis of microglial activation/innate immune response, astrogliosis as well as brain recovery. These novel model-systems represent a powerful analytical tool for understanding of *in vivo* pathology as well as for evaluating pharmacokinetics and longitudinal responses to drug therapies.

Wednesday AM - Plenary Session 1

Pharmacological Neurophysiology

All Roads to Psychosis Pass Through Dopamine Supersensitivity and Elevated D2High Receptors

Philip Seeman, M.D., Ph.D.; Departments of Pharmacology and Psychiatry, University of Toronto

Risk factors for psychosis, such as gene mutations, street drugs, birth injury, or trauma, can lead to compensation, with over-compensation leading to mental illness. The compensation involves synapses becoming supersensitive for adaptation and repair, protecting the brain from further injury. Individuals with schizophrenia are generally supersensitive to dopamine-like drugs such as amphetamine or methyphenidate. The heightened neurotransmission may be experienced subjectively as overstimulation, with attempts to psychologically adapt.

The dopamine D2 receptor is the common target for antipsychotics, and the antipsychotic clinical doses correlate with their affinities for this receptor. Antipsychotics quickly enter the brain to occupy 60-80% of brain D2 receptors in patients, with clinical improvement within days. The fast-off-D2 drugs clozapine and quetiapine do not elicit parkinsonism or tardive dyskinesia because they are released from D2 within hours.

The D2 receptor can exist in a state of high sensitivity for dopamine (D2High) or in a state of low sensitivity for dopamine (D2Low). All animal models of psychosis reveal elevations in D2High receptors, which is related to behavioural dopamine supersensitivity. These models include brain lesions, sensitization by drugs (amphetamine, phencyclidine, cocaine, corticosterone), birth injury, social isolation, and gene deletions in pathways for NMDA, dopamine, GABA, acetylcholine, and noradrenaline.

These multiple abnormal pathways pass through a final common pathway of dopamine supersensitivity and elevated D2High receptors, leading to psychotic signs. Future work will need to find a ligand that selectively labels D2High. The attempts by an individual to compensate for the brain injury with dopamine supersensitivity may lead to the signs and symptoms of psychosis.

Philip Seeman

Dr. Seeman was born in Winnipeg, Canada. He received a B.Sc. and an M.D. from McGill. He received a Ph.D. in Life Sciences in 1966, working with Dr. George Palade (1974 Nobel Laureate, Medicine) at Rockefeller University. Since 1967 he has been at the University of Toronto, Department of Pharmacology, and served as its Chairman (1977 – 1987). He is cross-appointed as a Professor of Psychiatry, and has held the University's Tanenbaum Chair in Neuroscience. His work on the membrane actions of drugs led to his discovery of the antipsychotic receptor, now known as the dopamine D2 receptor, the target for all antipsychotics. This research forms an experimental basis for the dopamine hypothesis of schizophrenia. Dr. Seeman and his research group, including H.B. Niznik, H. Van Tol and R. Sunahara, cloned three dopamine receptors: D1, D4 and D5. He has trained over 100 graduate students and Fellows. He has received 25 awards in recognition of his research.

Wednesday AM - Plenary Session 2

Clinical Practice Guidelines for Pharmacogenetic Testing: The First Six Drugs

MapQuest for Pharmacogenetic Tests: How to Arrive at Improved Clinical Outcomes

Larry Lesko, Department of Pharmaceutics, University of Florida

Larry Lesko

Dr. Lawrence J. Lesko is Professor of Pharmaceutics and Director of the Center for Pharmacometrics and Systems Pharmacology in the University of Florida College of Pharmacy at Lake Nona (Orlando). Dr. Lesko was Director of the Office of Clinical Pharmacology in the Center for Drug Evaluation and Research at the Food and Drug Administration for 16 years until his retirement in July 2011. At the FDA, Dr. Lesko led the advancement of personalized medicine through the update of labels of previously approved drugs with new genetic information. Dr. Lesko co-authored numerous Guidance for Industry including "Clinical Pharmacogenomics: Premarketing Evaluation in Early Clinical Trials" and "Pharmacogenomics Data Submissions". He also started a Division of Pharmacometrics that is responsible for quantitative analysis of dose-response and PK-PD relationships to support drug labels and NDA approvals, and the development of drug-disease models to support clinical trial simulations. Dr. Lesko led a program involved with mechanistic approaches to understanding drug safety including the prediction of off-target effects using bioinformatics approaches. Dr. Lesko has published more than 200 peer-reviewed manuscripts and is a frequent invited national and international speaker. In 2011, he received the Gary Neil Prize for Innovation in Drug Development from the American Society of Clinical Pharmacology and Therapeutics (ASCPT). Dr. Lesko also was honored with the Rawls-Palmer Progress in Medicine award from ASCPT, the Coriell Scientific Leadership Award for Personalized Medicine, the University of North Carolina Institute for Pharmacogenomics and Individualized Therapy Award for Clinical Service, and the Nathanial B. Kwit Distinguished Service Award for Clinical Pharmacology from the American College of Clinical Pharmacology. His hobbies include riding motorcycles and underwater photography. He is a Divemaster certified by the Professional Association of Dive Instructors.

Combined Abstract: U. Amstutz, P. Madadi, C Ross, R Jimenez, K Shaw, B Carleton

Our knowledge on genetic factors affecting drug effectiveness and the risk of adverse drug reactions is continuously increasing. Whereas this growing body of evidence has resulted in more pharmacogenomic markers being included in drug labels, the number of pharmacogenetic tests that are routinely used in clinical practice has remained small. An important factor in this slow clinical uptake of pharmacogenetic testing is the lack of clinical practice guidelines (CPGs) that provide health care providers with clear recommendations on the use and interpretation of genetic tests. To address this gap, a multi-disciplinary team of clinical pharmacologists, physicians, clinical geneticists, and pediatricians is developing evidence-based CPGs for six drugs (anthracyclines, carbamazepine, cisplatin, codeine, tamoxifen, warfarin) and their pharmacogenomic markers. Based on a systematic literature search, evidence on pharmacogenetic interactions was summarized and critically appraised using standard criteria. A simple set of clinical practice recommendations on (1) which patients should be tested and when, and (2) what therapeutic options should be considered for a given genetic test outcome were developed based on team consensus. Comprehensive CPG documents were created for each drug, containing background information on relevant clinical outcomes and pharmacogenetic interactions, concise summaries of scientific evidence, and clinical practice recommendations. In this session, the developed guidelines will be

presented, and meeting participants are invited to provide feedback as part of the guideline review process, in order to optimize the developed documents for clinical uptake, and ultimately to promote better-informed therapeutic decision-making that best fits the needs of the individual patient.

Ursula Amstutz
Dr. Ursula Amstutz is a Post-Doctoral Fellow with the Pharmaceutical Outcomes Programme at the University of British Columbia in Vancouver, Canada, and with the Canadian Pharmacogenomics Network for Drug Safety (CPNDS). She was trained in biology and biomedical sciences at the University of Bern, Switzerland. She obtained her MSc in Biology in the Division of Molecular and Computational Population Genetics, and her PhD in Cellular and Biomedical Sciences at the Institute of Clinical Chemistry at the Bern University Hospital. The main focus of her research is the investigation of genomic biomarkers for severe adverse drug reactions with the goal to make drug therapies safer for all patients. In particular, her current work addresses the study of genetic risk factors for serious drug-induced skin reactions, and the development of guidance tools to facilitate the incorporation of genetic information into clinical decision-making.

Parvaz Madadi
Dr. Parvaz Madadi is a Research Fellow in the Division of Clinical Pharmacology and Toxicology at the Hospital for Sick Children in Toronto. She holds a CIHR Postdoctoral Fellowship for her work with the Ontario Coroner's Office. She has also held Fellowship awards from the Canadian Pharmacogenomics Network for Drug Safety and the Quebec Training Network for Perinatal Research. Dr. Madadi received a Doctorate in Clinical Pharmacology and Toxicology from the University of Western Ontario in 2009.

Colin Ross
Dr. Colin Ross is an Assistant Professor in the Department of Pediatrics at the University of British Columbia at the Child and Family Research Institute (CFRI) at the BC Children's Hospital in Vancouver. Dr. Ross's research seeks to improve the lives of children by developing new tools for safer and more effective health care based upon an individual's genetic blueprint using the tools of molecular genetics, genomics, and personalized medicine. The debilitating and sometimes lethal consequences of severe adverse drug reactions (ADRs) are a striking problem in modern medicine. The consequences for patients who experience severe ADRs can be catastrophic. Together with Dr. Michael Hayden and Dr. Bruce Carleton, Dr. Ross helped found the 'Canadian Pharmacogenomics Network for Drug Safety' (CPNDS), a Canada-wide network of clinicians and researchers in 13 pediatric teaching hospitals to identify patients that have suffered severe ADRs. The overarching goal of this research is to identify the genetic and/or clinical factors of severe ADRs so that in the future these ADRs could be prevented by identifying those patients at greatest risk before the drug is administered.

Ricardo Jimenez
Dr. Ricardo Jimenez obtained his MD degree from Universidad La Salle, his specialty in Pharmaceutical Medicine at the National Polytechnic Institute, and his PhD in Pharmacology at the Center for Research and Advanced Studies, in Mexico City. He has been a fellow at the University of Miami, as well as scientist at the National Institute of Public Health in Mexico. At his previous appointment as a Medical Manager for a large pharmaceutical company, he developed and conducted clinical and epidemiological research in Latin America. Through this appointment, he had the opportunity to gain thorough knowledge of the health system structure in different countries, and implement avant-garde research in pharmacovigilance. Currently, he is expanding his research acumen as a postdoctoral fellow in outcomes research, especially in pharmacoepidemiology, drug utilization and pragmatic trials, in the Pharmaceutical Outcomes Programme at the University of British Columbia,

Kaitlyn Shaw
Kaitlyn Shaw is a Master of Science student at the University of British Columbia. She is currently completing her thesis project under the supervision of Dr. Bruce Carleton as a trainee in the CPNDS program, studying the pharmacogenetics of warfarin safety and effectiveness in children. She received her undergraduate degree in Pharmacology from the University of Western Ontario.

Bruce C. Carleton

Dr. Carleton's lifelong goal is to make medication use more effective and safer for all patients, particularly children. His research focus is on the impact of drug therapy on human health and quality of life. He is particularly interested in developing better ways to evaluate the effectiveness of drugs, medication-use models designed to improve patient health, as well as practical surveillance systems to improve the safe use of medication.

A key element of Dr. Carleton's research is the communication of results to clinicians, patients, healthcare administrators, and government officials – those who also hold responsibilities to improve patient care and our systems of healthcare delivery.

Dr. Carleton earned his Bachelor degree in pharmaceutical sciences in 1986 from Washington State University. He continued at the University of Utah, earning his Doctorate in Pharmacy degree in 1989. After completing a Residency in Clinical Therapeutics at the University of Utah and Primary Children's Medical Centers, he accepted a post-doctoral Fellowship at the University of Minnesota focusing on Experimental Therapeutics. Dr. Carleton completed a second Fellowship in Immunopharmacology at the University of Minnesota Transplant Center, and then joined the faculty at the University of British Columbia in 1991.

In addition to his appointment as Professor of Paediatrics and Co-Chair of the Division of Translational Therapeutics, Department of Pediatric Medicine at UBC, Dr. Carleton is a Senior Clinician Scientist at the Child & Family Research Institute. He directs the Pharmaceutical Outcomes Programme at BC Children's Hospital and he has served in this capacity since 1994. He holds appointments at UBC in the Centre for Health Services and Policy Research, the School of Population & Public Health and the Faculty of Pharmaceutical Sciences and the School of Health Information Science, University of Victoria. Dr. Carleton's public service is expansive. It includes serving as a charter member on the national Canadian Expert Drug Advisory Committee. Dr. Carleton was recently asked to serve the US Government as a Special Government Employee to advise the Advisory Committee for Pharmaceuticals and Clinical Pharmacology of the FDA.

Wednesday PM - Track 1

Alzheimer's Disease

The Role of AMPAR Trafficking in Memory Deficits Observed in Mouse Models in AD

Sheena Josselyn; Senior Scientist Neurosciences & Mental Health Sick Kids Research Institute; Associate Professor Department of Physiology, University of Toronto; Canada Research Chair, Molecular and Cellular Cognition

Alzheimer's disease (AD) is the most common form of dementia in the aging population yet there are few effective treatments for this devastating disorder. Although the precise molecular underpinnings of AD pathogenesis remain elusive, the majority of evidence indicates that β amyloid (Aβ a cleavage product of amyloid precursor protein (APP), is of primary importance. Although Aβ plaques are a hallmark of AD, loss of synapses is best correlate of memory dysfunction in the early stages of this disease. Moreover, there is growing evidence that Aβ disrupts synaptic function. For instance, Hsieh et al. (*Neuron*, 2006) showed that Aβ depresses excitatory synaptic transmission and decreases dendritic spine density in organotypic hippocampal slice cultures by removing postsynaptic AMPARs. Importantly, the endocytosis of postsynaptic AMPAR is also critical for some types of LTD. These findings are intriguing as they provide a link between the synaptic deficits associated with Aβ and the well-studied molecular pathways mediating synaptic plasticity. Furthermore, these *in vitro* observations suggest that some of the memory impairments observed in AD patients may be due to internalization of postsynaptic AMPARs, paralleling LTD. Although many important insights into the pathogenic mechanisms underlying AD have been gained by studying "AD in a dish", we believe that the definitive test of a potential mechanism/ treatment involves examining memory in behaving animals. Our lab directly tested whether the memory deficits observed in two mouse models of AD could be attributed to abnormal AMPAR trafficking. Our findings provide compelling evidence that targeting AMPAR may be a promising therapeutic strategy.

Sheena Josselyn
Dr. Josselyn is a Senior Scientist at the Hospital for Sick Children and an Associate Professor in the depts. of physiology and IMS at the University of Toronto. She is a Canada Research Chair in Molecular and Cellular Cognition and an EJLB Scholar. Her undergraduate degrees and a Masters degree in Clinical Psychology were granted by Queen's University in Canada. She received a PhD in Neuroscience/Psychology from the University of Toronto. Following this, she conducted post-doctoral work in Mike Davis's lab at Yale University and Alcino Silva's lab at UCLA. Her program of research is dedicated to understanding the neural basis of cognitive function and dysfunction. To unravel the molecular, cellular and circuit processes that underlie learning and memory, her lab uses a multidisciplinary approach including the use of genetically-engineered mice, viral vectors, cellular imaging, electrophysiology and detailed behavioral analysis.

Molecular Mechanisms Regulating the Abeta Amyloid Production

Weihong Song, Canada Research Chair in Alzheimer's Disease; Jack Brown and Family Professor; Director, Townsend Family Laboratories; Dept. of Psychiatry, Faculty of Medicine University of British Columbia

Alzheimer's Disease (AD) is the most common neurodegenerative disorder leading to dementia. Deposition of amyloid β protein (Aβ) to form neuritic plaques in the brains is the pathological hallmark of Alzheimer's disease (AD). Aβ is generated from sequential cleavages of the β-amyloid precursor protein (APP) by the β- and γ-

secretases. Beta-site APP cleaving enzyme 1 (BACE1) is the β-secretase essential for Aβ generation. Increased Aβ levels could facilitate AD pathogenesis and inhibition of Aβ generation may have therapeutic implications for AD treatment. Our studies showed that regulation of BACE1 expression plays an important role in AD pathogenesis and could be a valid target for AD drug development. We found that BACE1 tightly controlled APP processing and Aβ production in normal condition, and selection of β-secretase cleavage site by BACE1 had a dramatic effect on Aβ production in the pathological condition. Upregulating BACE1 expression by hypoxia facilitated neuritic plaque formation and potentiated behavioral deficits in AD pathogenesis. Furthermore, we found that GSK3β signaling regulated BACE1 gene expression and AD pathogenesis and that inhibition of GSK3 signaling reduced Aβ neuropathology and alleviate memory deficits in AD model mice.

Weihong Song

Dr. Weihong Song was trained as a clinical psychiatrist and molecular neuroscientist. He is a world-recognized expert studying the molecular and cellular mechanism of Alzheimer's disease pathogenesis and its drug development. Dr. Song was admitted to medical school at age of 14 in 1978 and received his medical degree from Chongqing Medical University in 1983. He completed his psychiatry residency and research program with a Master of Medicine degree at West China University of Medical Sciences in 1986. He worked as a chief resident and then as Chief Psychiatrist and a Lecturer of Psychiatry at West China University of Medical Sciences. Dr. Song received a MSc in psychobiology and behavioral genetics from Purdue University in 1993 and a PhD in medical neurobiology and molecular genetics from the Department of Psychiatry at Indiana University School of Medicine in 1996. He received his postdoctoral training in the Department of Neurology at Harvard Medical School and the Children's Hospital (1996-99), and worked as an Instructor at Harvard Medical School (1999-2001).

Dr. Song currently is a Full Professor with tenure and Canada Research Chair in Alzheimer's disease at UBC Department of Psychiatry. He holds the Directorship of Townsend Family Laboratories since 2007 and has been the endowed Jack Brown Family Professorship since 2001. He is also the Special Advisor to UBC President on China since January 2009. His lab has made major contribution to the understanding on how BACE1 and γ-secretase regulate APP processing and their role in Alzheimer pathogenesis and drug development. His recent work also defined the molecular pathways contributing to AD pathogenesis in Down Syndrome. He currently serves as a member of the editorial board of a prestigious medical journal "Journal of Clinical Investigation" and a Handling Editor of "Journal of Neurochemistry". Dr. Song has made major contribution in forging crucial Canada-China collaborations on research and education. He played an instrumental role in facilitating the establishment of the China-Canada Joint Health Research Initiatives, a funding program that is jointly managed and funded annually by the Canadian Institutes of Health Research (CIHR) and the National Natural Science Foundation of China (NSFC) since 2005. He also facilitated the collaboration of education between Canada and China including faculty exchange and high quality personnel training at postdoctoral, graduate and undergraduate levels. He has received numerous awards including the 2011 "Friendship Award", China's highest honor for foreign experts.

Caspases as Causative Agents in Alzheimer's Disease

Andrea Leblanc, Researcher, Bloomfield Centre for Research in Aging, Lady Davis Institute for Medical Research; James McGill Professor, Department of Neurology and Neurosurgery, McGill University

Caspase-6 (Casp6) is a cytosolic cysteinyl protease that is activated by exo-caspase or self-processing. Activation of Casp6 in human primary neurons increases the production of amyloid beta peptide, cleaves several proteins of the cytoskeleton such as Tau, tubulin, cofilin, actinins, impairs the ubiquitin-proteasomal system by cleaving p97, and cleaves a number of actin-regulating proteins localized to post-synaptic densities. The action of Casp6 induces axonal degeneration. We have found that Casp6 is highly activated in neuritic plaques, pre- and mature neurofibrillary tangles, and neuropil threads at the early and late stages of Alzheimer Disease. Active Casp6 is observed in both sporadic and familial forms of Alzheimer Disease. Furthermore, while active Casp6 is not observed in young human brains from 13 to 45 years of age, it is observed in the entorhinal cortex, the first brain area to be affected by Alzheimer Disease, of some non-cognitively

impaired aged individuals and the levels appear to correlate with lower cognitive abilities tested within a year of death. The amount of active Casp6 in brain and cerebrospinal fluid correlates with the level of dementia in Alzheimer Disease. Over-expression of a self-activated form of human Casp6 in the mouse hippocampal CA1 region of the brain results in age-dependent neuronal degeneration, inflammation, and memory impairment. These results indicate that Casp6 activation is upstream of several pathological and clinical features of Alzheimer Disease. Because Casp6 is activated pre-clinically, inhibiting the enzyme may prevent the progressive degeneration and dementia associated with Alzheimer Disease. We therefore propose that Casp6 is a novel therapeutic target of Alzheimer Disease

Andréa LeBlanc

Dr. LeBlanc is a James McGill Professor in the Department of Neurology and Neurosurgery at McGill University and a researcher at the Bloomfield Center for Research in Aging in the Lady Davis Institute for Medical Research in Montreal. Dr LeBlanc is also an associate member in the Department of Anatomy and Cell Biology and in the Division of Geriatric Medicine at McGill University. Dr LeBlanc received a Ph.D. in the Department of Biochemistry at Dalhousie University and did her post-doctoral training in the Department of Neurology at the Mayo Clinic and Mayo Foundation in Rochester, MN. Dr LeBlanc initiated her studies on Alzheimer and prion diseases as an assistant professor in the Department of Pathology at Case Western Reserve University in Cleveland, Ohio from 1989 to 1993. Dr LeBlanc is renowned for her discovery of Caspase-6 as a very early event in Alzheimer disease and her work on the neuroprotective function of normal cellular prion protein. The prion work was chosen as one of Quebec Science Top 10 discoveries in 2003. Dr LeBlanc has been a member of several committees at the National Institutes of Health and was appointed on their College of Scientific Review. She has been a chercheur boursier and a Chercheur National of the Fond de Recherche en Santé du Québec. In 2009, she was the first woman to receive an Honoris Causa Doctorate es Science attributed from the University of Moncton.

Neurotrophin Regulation and Cognitive Impairment in Alzheimer's Disease

Margaret Fahnestock, Professor, Psychiatry & Behavioural Neurosciences; Associate Director, Graduate Program in Neuroscience, McMaster Institute for Neuroscience Discovery & Study (MINDS)

How Alzheimer pathology produces memory loss is unclear. The neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are dysregulated in Alzheimer's disease and contribute to cognitive deficits in different ways. Increased proNGF, the precursor to NGF, and decreased BDNF correlate with the degree of dementia in Alzheimer's disease and mild cognitive impairment, and both NGF and BDNF rescue memory deficits in Alzheimer animal models. Therefore, neurotrophin dysregulation is thought to be a downstream mediator of cognitive impairment.

ProNGF is increased in Alzheimer's disease partly as a result of reduced retrograde axonal transport by basal forebrain cholinergic neurons. Coupled with the loss of its receptor, TrkA, proNGF accumulates and switches from neurotrophic to apoptotic activity, contributing to neurodegeneration. Amyloid-β reduces expression and signaling of BDNF, resulting in reduced synaptic plasticity. Amyloid-β down-regulates specific BDNF transcripts partly by reducing CREB phosphorylation. Together, these dysregulated neurotrophins compromise the survival and function of neurons critical for learning and memory.

Using a canine model of age-related cognitive impairment, we studied methods of increasing endogenous BDNF levels and cognition. Aged dogs exhibit reduced BDNF levels, increased amyloid-β levels and cognitive impairment, whereas the combination of an antioxidant diet and environmental enrichment raises BDNF to levels approaching those in young dogs, reduces amyloid-β levels and enhances cognition. Thus, changes in lifestyle in advanced age can increase BDNF to levels approaching those in the young brain and improve cognitive performance.

Margaret Fahnestock

Dr. Fahnestock is a Professor in the Department of Psychiatry & Behavioural Neurosciences at McMaster University, where she has been on the faculty since 1991. She received her B.Sc. (Honours) in Biological Sciences from Stanford

University and her Ph.D. in Biochemistry from the University of California at Berkeley. She did postdoctoral work in Cell Biology at Baylor College of Medicine and in Neurobiology with Eric Shooter at Stanford University. She worked at Stanford Research Institute (SRI International) from 1984-1991 and was a Visiting Scientist in the Department of Neurology at University of California at San Francisco from 1990-1991 before moving to Canada.

Professor Fahnestock's research is in the area of molecular neuroscience. She focuses on regulation of neurotrophic factor expression, particularly in neurological disorders such as Alzheimer's disease. She has published over 70 papers and 18 book chapters, has received funding from the NIH, MRC, CIHR, Alzheimer's Association (USA) and other foundations, and has served on numerous Canadian and U.S. grant committees.

She is active in the local chapter of the Society for Neuroscience (President three times of the Southern Ontario Neuroscience Association) and is co-founder and former Associate Director of McMaster's new Graduate Program in Neuroscience.

Early Inflammation and NGF Deregulation in Alzheimer's and MCI

Claudio Cuello; Department of Pharmacology and Therapeutics, McGill University

Alzheimer's disease (AD) has a "silent" phase which might last decades. The AD pathology is well advanced at the stage of Mild Cognitive Impairment (MCI), in which the brain already presents the classical hallmarks of AD. Current clinical trials have found no effective treatment or cure which might indicate that the therapeutic "time-window" should be sought at earlier stages.

We are investigating this in our rat and mouse transgenic models of AD which display biochemical, morphological and behavioral alterations in pre-plaque stages, equivalent to a preclinical phase of the human disease.

At this pre-plaque stage we have observed an abnormal intraneuronal accumulation of oligomeric and prefibrillar forms of Abeta peptides. This accumulation was paralleled by pro-inflammatory reactions such as elevated inflammatory markers and activated microglia mobilized to Abeta-burdened neurons.

We have reported the existence of an NGF (Nerve Growth Factor) metabolic pathway explaining the mechanisms for its maturation and degradation in the CNS. Our investigations in human brains revealed alterations in this metabolic pathway in AD and MCI compromising the availability of the mature form of NGF. Importantly, these alterations were also found in our animal models and were related to inflammatory processes.

We hypothesize that inflammation and alterations in NGF's metabolism are among the earliest events related to Abeta-driven toxicity in AD. These processes could offer new pharmacological targets as well as opportunities for earlier diagnosis and/or prevention of the disease.

This research is supported by the CIHR MOP 102752.

Claudio Cuello

Dr. Cuello is the Charles E. Frosst/Merck Chair in Pharmacology and past Head of the Department of Pharmacology and Therapeutics at McGill University, Montreal, Canada. He is an Adjunct Professor in Neuropharmacology at the Scripps Institute, La Jolla, CA and a visiting professor at Departments of Pharmacology and Pathology at Oxford University.

He leads a multidisciplinary research team working on brain aging and cellular and molecular neuropathology of Alzheimer's disease. Dr Cuello is an author of more than 300 peer reviewed scientific publications, has edited several books in his field and is on the editorial boards of numerous journals in the Neurosciences and Pharmacology, including TIPS. He is a past Staff Scientist of the Cambridge MRC-NCP Unit and past Professor in Neuropharmacology at Oxford University. Dr. Cuello graduated in Medicine in 1965 from the University of Buenos Aires, Argentina and in 1986 was granted a D.Sc degree by Oxford University for outstanding contributions to Neuroscience. He is recipient of numerous national and international prizes and holds several Honorary Doctorates.

Dr. Cuello has been named "Highly Cited Neuroscientist" by the Institute of Scientific Information (USA), is an elected Fellow of the Royal Society of Canada where he has served as Chair of the McLaughlin Medal Committee.

For his contributions in neuroscience, he was recently named an Officer of the Order of Canada.

Wednesday PM - Track 2

Cancer Stem Cells: New Potential Clinical Targets

SPONSORED BY: FMC BIOPOLYMER

Basic and Translational Biology of Cancer Stem Cells

William Matsui, M.D., The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, MD USA

Emerging data suggest that cells within an individual tumor are both phenotypically and functionally heterogeneous despite their clonal origins. In many malignancies, most cells appear to lack significant replicative potential and instead arise from relatively rare populations of phenotypically distinct cancer stem cells. In both hematologic malignancies and solid tumors cancer stem cells have been prospectively identified based on their ability to give rise to differentiated progeny that recapitulates the original tumor in the ectopic setting. Additionally, these cells are capable of self-renewal and may be relatively resistant to various anti-cancer agents. These unique functional properties suggest that cancer stem cells play a central role in disease initiation, relapse, and progression and that the development of effective strategies inhibiting these cells may ultimately improve long-term clinical outcomes. We have studied multiple myeloma and found that the malignant plasma cells forming the tumor bulk and characterizing the disease arise from cancer stem cells phenotypically resembling normal memory B cells. Recently, we have translated these findings and initiated novel clinical trials explicitly designed to target myeloma stem cells. We will discuss the strategies used to identify novel cancer stem cell-targeting agents in the laboratory and develop biomarker strategies to monitor their efficacy in the clinical setting.

William Matsui
William Matsui joined the faculty of the Department of Oncology at the Johns Hopkins University School of Medicine in 2001 and is currently an Associate Professor in the Division of Hematologic Malignancies. He received his undergraduate degree in biochemistry from Harvard College in 1989 and his medical degree from the University of California at San Francisco in 1995. He completed his residency training in internal medicine at the University of Washington in Seattle and his clinical training in Medical Oncology at Johns Hopkins. The primary focus of Dr. Matsui's laboratory is examining the role of cancer stem cells in hematologic malignancies. More recently, his laboratory has expanded these studies to pancreatic adenocarcinoma and the development of novel strategies targeting cancer stem cells in the clinical setting.

Targeting Cancer Stem Cell-Specific Molecular Pathways

Markus Frank, Harvard Medical School; Transplantation Research Center, Brigham & Women's Hospital and Children's Hospital Boston, Boston, MA

The cancer stem cell (CSC) concept posits that malignant tumors, like many healthy tissues, can be hierarchically organized at the cellular level and that subpopulations of CSCs, representing the apex of such discernible hierarchies, are exclusively responsible for tumor initiation and propagation. According to the CSC model of tumor growth and progression, a CSC is defined as a cancer cell that possesses (1) a capacity for prolonged self-renewal that inexorably drives tumor growth and (2) a capacity for differentiation, that is, the production of heterogeneous lineages of daughter cells that represent the bulk of the tumor mass, but are

dispensable for tumor propagation. Consequently, CSCs are thought to lie at the root of primary tumor formation and growth and to be also responsible for tumor dissemination, metastasis formation, therapeutic resistance, and posttreatment recurrence. The rapidly expanding interest in CSCs in the field of oncology is based on their exceptional promise as paradigm-shifting novel targets for cancer therapy. Here, several potential strategies of CSC targeting will be discussed, based on recently identified CSC functions in human malignant melanoma.

Markus Frank

Markus Frank currently serves as Assistant Professor of Pediatrics and Dermatology at Harvard Medical School and as Associate Physician at Brigham and Women's Hospital. He is a graduate of Harvard University in the field of Biochemistry and a M.D. graduate of the University of Heidelberg School of Medicine. He received residency training in internal medicine at the Albert Einstein College of Medicine and fellowship training in nephrology and transplantation immunology at Brigham and Women's Hospital, Children's Hospital and Massachusetts General Hospital. Dr. Frank is a leading expert in the field of cancer stem cell biology and antibody-based cancer stem cell targeting strategies. His laboratory was the first to clone and characterize the multidrug resistance gene ABCB5, which identifies melanoma stem cells that can be therapeutically targeted to inhibit tumor growth, providing initial proof-of-principle for the potential therapeutic utility of the cancer stem cell concept. Dr. Frank is an elected member of the American Society for Clinical Investigation (ASCI).

Targeting Cancer Stem Cells: Promises and Challenges Ahead

Feridoun Karimi-Busheri, Nova Rx Corporation, and Department of Oncology, University of Alberta, Edmonton, AB

Overwhelming reports indicate that a subset of cells different from the rest of tumor cells, known as cancer stem cells, may be behind tumor formation and invasion. They share many similarities to normal stem cells and at the same time profound differences which separate them from each other. These cells are in an undifferentiated state capable of self-renewal and exhibit distinct patterns of gene expression. They are *equipped* with an inherent *array* of *defense* mechanisms to resist conventional therapies such as radiation and chemotherapy through the mechanisms of altered gene regulation during apoptosis, senescence, cell cycle, and DNA repair pathways. Drug resistance also is accomplished through the upregulation of multidrug-transport proteins in the cell membrane, such as ABCB1 and ABCG2 families, that occurs in normal stem cells as well. They have different immune evasion strategies and at the molecular level the signaling cascades, such as sonic hedgehog and Wnt, epidermal growth factor receptor, NOTCH, and polycomb gene product BMI-1 are deregulated in cancer stem cells. These paramount differences are ideal in designing various drugs capable of targeting different pathways. Moreover, further *evidence* is accumulating in support of an association between cancer stem cells and the epidermal to mesenchymal transition process, EMT, which is the driving force behind tumor progression and metastasis. Although there are many hurdles and unresolved issues, targeting cancer stem cells offers a new paradigm in cancer therapy that requires unique therapeutic strategies.

Feridoun Karimi-Busheri

Feridoun Karimi-Busheri is the Senior Investigator and Director of Stem Cell Department at NovaRx Corporation, a clinical-stage biopharmaceutical company in the United States with a lung cancer vaccine in the last few months of an accelerated Phase III Clinical Trial globally, including in Canada. Currently he is on sabbatical at the University of Alberta in Edmonton. His research is towards developing novel anti-cancer stem cells therapies, signaling pathways targeted therapy, biomarker discovery, and biobanking.

He is an involved member of various societies, including, but not limited to: The Canada Research Chairs Program College of Reviewers, honorary member of the Canadian Society for Pharmaceutical Sciences, International Society for Stem Cell Research, and the co-founder of The Biosocial Society, U.K. He is the associate editor of the Journal of Pharmacy & Pharmaceutical Sciences and serves on the review board of numerous professional journals and grant agencies. Dr. Karimi-Busheri has also widely published in many peer-reviewed journals, including Cell, Molecular Cells, PNAS, Journal of Stem Cells, and Cancer Research. Additionally, His Ph.D. work was published in Nature and was later the subject of a BBC Wales television program.

Wednesday PM - Track 3

The New Canadian BE Guidelines in the Midst of Current Challenges from a Regulatory and Industry Point of View

SPONSORED BY: BASF

The New Canadian Bioequivalence Guidelines

Eric Ormsby, Office of Science, Health Canada, Ottawa, Ontario

Canada first published its guidance on the conduct and analysis of bioequivalence studies in 1992. Since this time much experience has been gained and two new guidances have been finalised by Health Canada. It has combined bioequivalence guidances Part A and Part B as well as Report C recommendations from the Expert Advisory Committee. It has integrated issues from 8 notices on bioequivalence. This talk will outline the changes to Health Canada's approach to bioequivalence determination.

Eric Ormsby
Eric has worked for Health Canada for over 30 years, almost entirely in some form of what is now called the Therapeutic Products Directorate (TPD). The TPD is responsible for pre-market assessment of pharmaceuticals and medical devices. Eric has been involved in bioequivalence issues since 1986 when Canada first began to develop a regulatory framework for bioequivalence.

Eric obtained a BSc. in Genetics and Statistics from the University of Guelph and a MSc. in Biostatistics also from Guelph. Currently he is manager of the Office of Science in the Bureau of Policy, Science and International Programs of TPD. This Office has the responsibility of managing TPD=s access to external expert advice, managing the reconsideration process and the development of science based regulations, policies and guidelines.

Bioequivalence Review Challenges in a Changing Regulatory Environment

Jaigi Mathai, Bureau of Pharmaceutical Sciences, Therapeutic Products Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON

Resource and workload challenges have led to a recent change in the Health Canada's user fees structure and consequently the strategies with which the review of submissions packages are conducted. Modernisation is part of Health Canada's plan to ensure a sustainable regulatory future, which includes the exploration of various global initiatives and international partnering projects that may aide in addressing common challenges. Resultant pressures on time require that review efficiency be optimised, and several issues that are commonly encountered in the assessment of comparative bioavailability studies will be discussed in this talk. The adoption of European Medicines Agency Guideline on Bioanalytical Method Validation into the new Canadian Bioequivalence guidance will also be reviewed.

Jaigi Mathai
Jaigi obtained a BSc(H) in Biochemistry from Queen's University in Kingston, Ontario, and a Ph.D. from the department of Biochemistry at McGill University in Montreal, Canada. He was awarded the CIHR/TPD/RxD post-doctoral fellowship, and has been working at Health Canada for nearly seven years. Here Jaigi has conducted evaluations of both clinical and non-clinical submissions in the Oncology Division at the Therapeutic Products Directorate (TPD), the Office of Clinical Trials (TPD), the Oncology Unit at the

Biologic and Genetic Therapies Directorate (BGTD), and has been a Senior Assessment Officer in the Division of Biopharmaceutics Evaluation 2 (TPD) for the past three years. Jaigi was member of the working group that developed the 'Pharmacogenomics' guidance document and is currently a member of the TPD Good Review Practices Steering Committee.

Challenging Issues on the Demonstration of Bioequivalence

Yu Chung Tsang, Apotex Inc., Toronto, Ontario, Canada

Bioequivalence has been the traditional methodology for proving therapeutic equivalence of a new or modified formulation of a drug product. It is also commonly used for ensuring that a generic product is equally safe and efficacious as the brand product, thereby allowing a cheaper alternative to be available to patients. To enable the realization of substantive saving, however, generics should not be over-burdened with unnecessary studies or requirements for the demonstration of bioequivalence. The criteria for bioequivalence should not require an extremely large number of subjects and/or should not be so stringent that even the brand product would have trouble passing against itself. While the current bioequivalence requirements has been working relatively well for the regulatory approval of generic product for uncomplicated drugs, including those with modified release formulation, there are still challenging issues that need attention from the regulatory authorities. Health Canada should be commended for revising their bioequivalence guidelines to tackle the challenges evolved from increasing complexity of new drug products. The impact of some of the changes in the new guidelines on the demonstration of bioequivalence will be discussed in the presentation. It is noteworthy that the effort should not stop here as there are still changes that could result in significant reduction of unnecessary burden in demonstrating bioequivalence. One example is the expanded use of biowaiver for different strengths of a drug product or for drugs that belong to Class I of the Biopharmaceutics Classification System. In addition, other challenging issues that have not been tackled in the new guidelines will be brought up for discussion. It is important that regulatory authorities work with the scientific community and drug industry to come up with solution to these challenging issues.

Yu Chung Tsang

Dr. Yu Chung Tsang is currently working at Apotex Inc. as Chief Scientific Officer, Biopharmaceutics and Biostatistics. He obtained his bachelor degree (1984) in Pharmacy and Ph.D. degree in the area of Pharmacokinetics in 1990 from the University of Toronto. He has been with Apotex since then. His main responsibility is to provide scientific expertise and strategic direction in the design of bioequivalence studies and the analysis of data for the development of pharmaceutical products in the Apotex group of companies. To date, he has been involved with the design and data analysis of over a thousand bioequivalence studies for the registration of over 200 drugs in Canada, US, EU and many other international marketplaces. He also provides statistical support in clinical trials of new chemical entities at Apotex. Dr. Tsang is currently the Chair of the Bioequivalence Committee in the Canadian Generic Pharmaceutical Association. He is also a member of the Bioequivalence Working Group of the European Generic Medicines Association, and a Steering Committee member for the Generic Pharmaceuticals and Bioequivalence Focus Groups of the American Association of Pharmaceutical Scientists. He has been a member of the Board of Directors for the Canadian Society of Pharmaceutical Sciences from 2008-2010. Aside from his industrial experience, he also holds an appointment (status only) at the Leslie Dan Faculty of Pharmacy, University of Toronto.

Wednesday 4:00 PM

CSPT Senior Investigator Award Lecture

From Drugs to Patients: Personalizing Care, not Medicines

Bruce Carleton, Director, Pharmaceutical Outcomes Programme, BC Children's Hospital.; Senior Clinician Scientist, Child & Family Research Institute, Vancouver.; Senior Clinician Scientist, CFRI; Professor, Department of Pediatrics, University of British Columbia; Director, Pharmaceutical Outcomes Programme, BC Children's Hospital

I started my scientific career with a focus on developing a deeper understanding of the pharmacology of immunomodulating drugs. Over time, I began to better appreciate the importance of a career focused on patient outcomes from drug therapy . In the end, there are only two reasons to use drugs in the universe - prolong life or improve quality of life. These two goals of therapy are extremely difficult to assess in clinical practice. The same drug therapy produces a heterogeneity of drug responses in patients. So the development of specific assessment tools to meet not "drug therapy goals" but rather "therapeutic objectives" became my raison d'etre. I then encountered a young child who died as a result of ibuprofen exposure - clearly an effective drug and one that is quite safe for use in the majority of patients. Her grieving parents' faces are permanently etched in my memory. What tools could we develop to predict these types of idiosyncratic reactions and avoid them? While working at BC Children's Hospital as a 'drug expert' I needed to at least consider this question which kept me awake many nights. My talk will highlight my path from budding clinician scientist to the present. Clinicians and scientists will likely find humour in my challenges and failures along this path. Trainees may find this helpful as they develop their own career trajectories and see these paths change (often without expectation) over time. I had no idea I would end up here. Sir Winston Churchill perhaps said it best, "Success is the ability to go from one failure to another with no loss of enthusiasm". And my failures won't stop - there is much more to do. But nor will my enthusiasm for finding the best ways to make medication safer and more effective for children. I hope you enjoy the journey as much as I am.

Bruce Carleton
See Bio on page 35.

Thursday 8:00 AM - Track 1

Food Intake and Reward

Effects of Insulin on VTA Dopamine Neurons

Stephanie Borgland, Department Anesthesiology, Pharmacology & Therapeutics, University of British Columbia

The prevalence of obesity has drastically increased over the last few decades. Exploration into how hunger and satiety signals influence the reward system can help us to understand non-homeostatic mechanisms of feeding. Previous research has implicated mesolimbic dopamine signaling in the incentive, reinforcing, and motivational aspects of food intake. Insulin receptors are expressed in dopaminergic neurons of the ventral tegmental area (VTA) and there is substantial evidence suggesting that insulin may act in the VTA to suppress feeding. However, the neural mechanisms underlying insulin effects in the VTA remain unknown. We demonstrate that insulin can cause a long-term depression (LTD) of excitatory synapses onto VTA dopamine neurons. This effect requires endocannabinoid-mediated presynaptic inhibition of glutamate release. Using fast scan-cyclic voltammetry to measure subsecond dopamine concentrations in the VTA, we found that insulin dose-dependently reduced dopamine concentration by increasing the reuptake of dopamine through its transporter. Taken together, these results demonstrate that insulin acts in the VTA to depress excitatory synaptic transmission of dopamine neurons as well as somatodendritic dopamine concentrations. Furthermore, insulin action in the VTA may serve to reduce the desire to eat.

Stephanie Borgland
The goal of my research is to understand the synaptic and cellular mechanisms occurring in the mesolimbic dopaminergic system underlying the motivation to consume food or drugs of abuse. I obtained my PhD from the University of Sydney, Australia in 2002. I worked as a postdoctoral fellow with Antonello Bonci from 2002-2006 and then as Associate Investigator in 2007 at the University of California, San Francisco. In 2008, I was appointed as an Assistant professor to the Department of Anesthesiology, Pharmacology and Therapeutics at the University of British Columbia

Negative Emotional States and Neurobehavioral Adaptations in Brain Reward Circuitry Induced by Chronic High-fat Feeding

Stephanie Fulton Montreal Diabetes Research Center, Centre de Recherche du CHUM, Université de Montréal

Palatable high-fat and high-sugar foods are rewarding and their consumption is associated with adaptations in brain reward circuitry. Dopamine neurons originating in the ventral tegmental area (VTA) and substantia nigra of the midbrain that innervate limbic sites including the nucleus accumbens (NAc) and dorsal striatum are an essential component of the neural circuitry underlying, emotion, motivation and reward. To understand the neural mechanisms by which obesity is linked to an increased risk of depressive and mood disorders we are studying the effects of chronic high-fat feeding on negative emotional states, depressive-like symptomology and food cravings in rodents. We found that high fat feeding and diet-induced obesity increases anxiety-like behavior, HPA reactivity and behavioral despair in mice in a manner that is positively associated with protein levels of brain-derived neurotrophic factor and phosphorylated CREB in the NAc and dorsal striatum. In addition, we find that prior chronic high-fat feeding in mice potentiates stress responses and palatable food cravings following diet withdrawal (dieting). Our data suggest that negative emotional states seen in obesity are a consequence of increased caloric intake and increased BDNF and pCREB levels in the striatum, and that saturated fat intake, in

particular, renders subjects more susceptible.

Stephanie Fulton
Dr. Fulton carried out her graduate training in behavioral neurobiology at Concordia University in Montreal under the mentorship of Peter Shizgal and Barbara Woodside. Her research led to the discovery that the adipose hormone leptin modulates brain reward circuitry. As a CIHR postdoctoral fellow in the laboratory of Jeffrey Flier at Harvard Medical School she and her colleagues identified the critical role of leptin in mesoaccumbens dopamine signaling and function. Additional postdoctoral training with Louis-Eric Trudeau led to the discovery of the role of Kv1 potassium channels in the regulation of striatal dopamine release. Stephanie became a principle investigator in 2008 at the Centre de Recherche du CHUM and Montreal Diabetes Research Center and Assistant Professor in the Departments of Nutrition and Physiology at Université de Montréal. Using rodent models, her lab studies how leptin and nutrients directly impact mesolimbic dopamine neurons to regulate energy balance, food-motivated behavior, anxiety and dopamine tone. Other objectives include exploring how chronic consumption of diets rich in a fat impact the neural circuitry regulating emotion and motivation.

Ghrelin Modulation of Dopamine Neurotransmission

Alfonso Abizaid, Associate Professor and Graduate Student Chair, Carleton University

Ghrelin is a 28 amino-acid peptide that was first identified as an endogenous ligand to growth hormone secretagogue receptors (GHSR). In addition to the hypothalamus and brain stem, GHS-R message and protein are widely distributed throughout the brain with high expression being detected in regions associated with goal directed behavior. Of these, the ventral tegmental area (VTA) shows relatively high levels of mRNA transcript and protein. Interestingly, ghrelin appears to be involved in appetitive feeding behaviors given that mice and rats with mutations to the ghrelin receptor gene show attenuated anticipatory responses to scheduled meals, and decreased hedonic feeding, in correlation with deficits in neuronal activation of hypothalamic and mesolimbic regions associated with food seeking behaviors. Moreover, direct delivery of ghrelin into the VTA of rats, results in increased operant responses for food on fixed and progressive ratio schedules of reinforcement. Based on these data, we propose that VTA dopamine cells, in parallel with peptidergic cells in the hypothalamus, are sensitive to changes in circulating levels of ghrelin and that these may alter the motivational state of mammals, making the more likely to engage in food seeking behaviors and perhaps in behaviors to obtain other reinforcers like sex or drugs of abuse.

Alfonso Abizaid
Dr. Abizaid obtained a PhD in Psychology (Behavioral Neuroscience) from Concordia University training under the supervision of Dr. Barbara Woodside studying the neuroendocrine regulation of energy balance across different reproductive states in female rats. I was fortunate to be sponsored by NSERC to conduct a postdoctoral fellowship in the Department of Gynecology and Obstetrics at the Yale University School of Medicine, where I began working on the neuroendocrine effects of ghrelin on food intake and energy metabolism under the supervision of Dr. Tamas Horvath. I have continued on this work during the past 5 years at Carleton University where I am now an Associate Professor in the Department of Neuroscience.

Co-sensitivity to the Incentive Properties of Palatable Food and Cocaine in Rats; Implications for Comorbid Addictions

Francesco Leri, Associate Professor, Department of Psychology, University of Guelph.

Several lines of evidence suggest that there may be a shared vulnerability to acquire behaviors motivated by strong incentive stimuli. Methods: Non-food restricted male Sprague-Dawley rats (n=78) underwent place conditioning with Oreos, and were subsequently tested on cocaine self-administration (SA) on fixed and progressive ratios, as well as extinction and reinstatement by cocaine primes and by consumption of Oreos. Results: Although there was a group preference for the Oreo-paired compartment, at the individual level some rats (69%) displayed a preference and others did not. In cocaine SA, "preference" rats achieved higher break points on a progressive ratio, and displayed greater responding during extinction and cocaine-induced reinstatement. Within the context of this study,

Oreo-cocaine cross-reinstatement was not observed. In a control study, rats (n=29) conditioned with a less palatable food (rice cakes) also displayed individual differences in place preference, but not on subsequent cocaine tests. Conclusions: These findings indicate that there is a relationship between incentive learning promoted by palatable foods and by cocaine. This supports the hypothesis that comorbid food-drug addictions may result from a shared vulnerability to acquire behaviors motivated by strong incentives.

Francesco Leri

Dr. Leri graduated McGill University, Montreal, Canada in 1999 with a PhD in the field of Psychology. He completed a post-doctoral fellowship at Concordia University, Montreal, Canada in 2001 in the field of Neurobiology. His expertise is behavioral neuropharmacology, and the majority of the published studies employed laboratory rats to explore particular behavioral and neurochemical aspects of substance dependence.

As an independent Investigator at the University of Guelph, my team and I have been developing three lines of research. The first explores neurobiological mechanism implicated in relapse to heroin seeking. The second involves the study of comorbidity between oxycodone/cocaine dependence and other Axis I & II disorders. The third involves the study methadone's effect on behaviors motivated by cocaine. In collaboration with Dr. Zhou and Dr. Kreek in the Laboratory of the Biology of Addictive Diseases at the Rockefeller University, we have also explored the effects of drugs of abuse on stress responsive systems, and we have developed a productive line of research examining neurochemical systems involved in the effects of methadone on cocaine seeking behavior.

A Psychogenetic Study of Food Addiction in Adult Men and Women

Caroline Davis Faculty of Health - School of Kinesiology & Health Science, York University

There is growing evidence of 'food addiction' (FA) in sugar- and fat-bingeing animals. The purpose of this study was to investigate the legitimacy of this construct in the human condition, and to extend the validation of the Yale Food Addiction Scale (YFAS) - the first tool developed to identify individuals with addictive tendencies towards food. Using a sample (n=90) of overweight and obese adults (aged 25-45 years), and a case-control methodology, we focused our assessments on three domains relevant to the characterization of conventional substance-dependence disorders: clinical co-morbidities, psychogenetic risk factors, and abnormal motivation for the addictive substance. Results were strongly supportive of the FA construct and validation of the YFAS. Those who met the diagnostic criteria for FA - based on the established American Psychiatric Association criteria for substance dependence - had a significantly greater co-morbidity with binge eating disorder, depression, and attention-deficit/hyperactivity disorder compared to their age- and weight-equivalent counterparts. They were also more impulsive, displayed greater emotional reactivity, and reported greater food cravings and 'self-soothing' with food compared to the obese controls. Finally, using multilocus genetic profiles with 4 functional dopamine polymorphisms, FA were characterized by stronger signaling in the brain's reward pathway, suggesting a stronger hedonic response to food than their non-FA counterparts. These findings advance the quest to identify clinically relevant subtypes of obesity that may possess different vulnerabilities to environmental risk factors, and thereby could inform more personalized treatment approaches for those who struggle with overeating and weight gain.

Caroline Davis

Dr. Davis has had 25 years of academic and research experience in the field of disordered eating behaviours. Her investigations have focused on the clinical and psychological vulnerability factors for these disorders – and more recently, on genetic and neuropsychological indices of risk. Her current work focuses on the strong parallels between compulsive overeating/binge eating disorder and more conventional addiction disorders like substance abuse. This work has highlighted the prominent links between overeating behaviours and Attention Deficit/Hyperactivity Disorder. She has published extensively on these topics in high-quality peer-reviewed journals, and has also disseminated her research as an invited speaker at numerous scientific conferences. In addition, she has attracted substantial external funding from CIHR and SSHRC.

Thursday 8:00 AM - Track 2

CSPS Award Lectures

Glaxosmithkline/CSPS Early Career Award Lecture

Non-invasive Drug Delivery – Rational Design and Characterization of Nanoparticulate Delivery Systems

Ildiko Badea, University of Saskatchewan, Saskatoon, SK

The application of biotechnology in medicine resulted in an explosion of new therapeutic agents, such as proteins, DNA, and RNA. The major advantage of these drugs over chemical agents is their specificity and selectivity. My research focuses on the use of DNA as a biotechnology drug. Similar to the majority of biological molecules, DNA is degraded rapidly in the body. Its encapsulation into a delivery system increases circulation time and permits accumulation of the drug in certain organs.

Thus, pharmaceutical formulation development is one of the key steps in the design and development of gene therapy products. Lipid-based soft nanoparticles and nanodiamond-based gene delivery systems, which have the ability to encapsulate or complex DNA and shuttle it into the cells are developed in my laboratory. We use state-of-the-art technology for the systematic evaluation of the gene transfer efficiency of these novel nanoparticles. Assessment of the DNA delivery systems will lead to the development of in vivo gene delivery systems for topical non-invasive administration.

The development of novel delivery systems has also given new hope for synthetic therapeutic agents that are effective but cannot reach their full potential in clinical evaluations due to the lack of selectivity and poor bioavailability. Incorporation of novel, poorly soluble anticancer drugs in delivery systems improves their pharmacokinetic profile and reduces their toxicity. In my laboratory, we evaluate the ability of the encapsulated drug to stop cell proliferation, and programmed cell death. The successful targeted delivery of the cytotoxic agents into cancer cells without affecting healthy cells will lead to applied research for cancers with poor outcomes, such as skin cancers.

Ildiko Badea

Dr. Ildiko Badea is an Assistant Professor of Pharmaceutical Sciences in the College of Pharmacy and Nutrition at the University of Saskatchewan since 2007. She obtained a B.Sc.Pharm. degree in Romania and worked as community pharmacist and clinical pharmacist for 10 years. Her Ph.D thesis in the College of Pharmacy and Nutrition, University of Saskatchewan (degree awarded in 2006), was based on the study of gemini surfactant-based gene delivery agents. Dr. Badea completed one year of postdoctoral fellowship at the Vaccine and Infectious Disease Organization.

Dr. Badea's research focuses on the design and characterization of nano-size drug delivery systems for encapsulating both small molecules and biotechnology drugs. She has published 26 refereed articles to date and contributed to two book chapters and a book. Her research is funded by the Canada Foundation for Innovation, Saskatchewan Health Research Foundation, NSERC, the College of Pharmacy and Nutrition and the Drug Design and Discovery Research Group. She is supervising two MSc and two PhD students along with undergraduate summer students.

She was invited for external review of NSERC grants and is a member of the Saskatchewan Health Research Foundation review panel. Dr Badea was invited to review manuscripts for several journals (Current Drug Delivery, Biochimica et Biophysica Acta, European Journal of Pharmaceutics and Biopharmaceutics, Journal of Drug Targeting, Journal of Pharmacy and Pharmaceutical Sciences, International Journal of Nanomedicine, Molecular Biotechnology, Physical Chemistry Chemical Physics)

She is involved in teaching at both undergraduate and graduate level, being responsible

for core curriculum subject Foundations of Pharmacy: Pharmaceutical Dosage Forms and graduate courses regarding nanomedicine.

Dr Badea received the Gattefossé Canada/CSPS Award in 2007 and the University of Saskatchewan Industry Liason Office Innovation Challenge Award.

CSPS Award of Leadership in Canadian Pharmaceutical Sciences

Leslie Dan, Teva Canada, Toronto, ON

Leslie Dan arrived to Canada in 1947, with ten dollars in his pocket, as a refugee. He worked as a lumberjack, waiter and tobacco picker allowing him to attend high school and university. He enrolled in the Faculty of Pharmacy of University of Toronto, graduating in 1954. He received an MBA at the university in 1959.

His first, inventive business, in the late 1950s, was to sendmedical parcels overseas. In 1961, hestarted an over-the-counter drug distribution company and, then in 1965, he founded Novopharm Ltd. Under his leadership, the company grew from one product, a generic version of tetracycline, to one of Canada's largest generic pharmaceutical manufacturers. By this time, it had sales of $750 million and employed 3,000 people, a far cry from the sales of $ 165,000 in the first year. He was chairman and CEO of the company until it was sold to Teva Pharmaceuticals in 2000.

In 1990, Dr. Dan founded Viventia Biotechnologies Inc., which specializes in the discovery and development of a new generation of immunotoxin products which can be applied to safer and more beneficial cancer therapies. He is currently chairman of the company.

The Canadian Medicine Aid program, which sends drugs to the Third World, was started by Dr. Dan in 1985. Every year, shipments worth millions of dollars are sent to doctors, pharmacists, nurses and charities working in developing countries.

Dr. Dan has received many awards for his contributions to the development of the Canadian pharmaceutical industry and pharmaceutical science. He is an Officer of the Orderof Canada and has received the Order of Ontario. Honorary doctorate degrees from the University of British Columbia, York University, University of Toronto, and Dalhousie University have been bestowed upon him.

The Award of Leadership he is now receiving is an award bestowed upon an individual who has demonstrated leadership in advancing the cause of pharmaceutical research and development in Canada.

Thursday 9:00 AM - Track 2

Advances in Research of Drug Transporters and Metabolic Enzymes: Implications in Drug Development

Yuichi Sugiyama, Sugiyama Laboratory, RIKEN Research Cluster for Innovation, RIKEN (The Institute of Physical and Chemical Research), Yokohama, Japan

Drug transporters are expressed in many tissues, such as the intestine, liver, kidney, and the brain, and play key roles in drug absorption, distribution and excretion. In this presentation, I will summarize the significant role played by drug transporters in drug disposition, focusing particularly on their potential use during the drug discovery and development process. The use of transporter function offers the possibility of delivering a drug to the target organ, avoiding distribution to other organs (thereby reducing the chance of toxic side-effects), controlling the elimination process, and/or improving oral bioavailability. It is useful to select a lead compound that may or may not interact with transporters, depending on whether such an interaction is desirable. The changes in pharmacokinetics due to genetic polymorphisms and drug-drug interactions involving transporters can often have a direct and adverse effect on the therapeutic safety and efficacy of many important drugs.

Even when drugs ultimately undergo metabolism, their elimination rate is sometimes determined by the uptake rate mediated by transporters. Elucidation of the rate-determining process in the overall hepatic elimination of drugs is therefore critical for predicting their hepatic clearance, and their systemic and regional exposures. I will show you how to establish a physiologically based pharmacokinetic (PBPK) model that includes the transporter-mediated membrane transport and enzyme-mediated metabolism processes and to investigate the effect of changes in transporter function on the pharmacokinetics and, ultimately, the pharmacological and/or toxicological effects.

(1) Giacomini KM and Sugiyama Y. Membrane transporters and drug response, in "*Goodman & Gilman's The Pharmacological Basis of Therapeutics 12th Edition*", (Brunton LL, Chabner BA, Knollman B, eds) Chapter 5, McGraw-Hill Companies, New York, NY, pp 89-122 (2011).

(2) Maeda K. and Sugiyama Y. In vitro-In vivo Scale-up of Drug Transport Activities. In: "Drug Transporters" ed. by You G. and Morris M.E., Wiley Interscience pp. 557-588(2007)

(3) Watanabe T, Kusuhara H, Maeda K, Shitara Y and Sugiyama Y Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. J Pharmacol Exp Ther 328:652-662 (2009).

Yuichi Sugiyama

Yuichi Sugiyama started working as the Head of Sugiyama Laboratory, RIKEN Innovation Center, RIKEN Research Cluster for Innovation, Yokohama, Japan since April 1, 2012. He has been the Professor, Department of Molecular Pharmacokinetics at the University of Tokyo since 1991, retired from the University of Tokyo in March, 2012 and moved to RIKEN.

His work is internationally recognized by prestigious awards, including AAPS Distinguished Pharmaceutical Scientist Award, 2003, Scientific Achievement Award 2004 from the

"Pharmaceutical Society of Japan(PSJ), and the PSWC (Pharmaceutical Sciences World Conference) Research Achievement Award in 2007**,** FIP Hoest Madsen Gold Medal in 2009, "Medal with Purple Ribbon" given by Government in 2010 and B.Brodie Award from ASPET in 2012. According to a recent report of ISI Essential Science Indicators, Professor Sugiyama has been ranked as the top (#1) cited scientist in the field of Pharmacology & Toxicology. His original articles published in the past 10 years, between January 1997 and February 2007, have received the highest number of citations in this field.

He served as the chairman of Board of Pharmaceutical Sciences in FIP (2000-2004). He was also the president of both "International Society for the Study of Xenobiotics (ISSX)" and "Japanese Society for Xenobiotic Metabolism and Disposition (JSSX)".(2006-2007).

Thursday 8:00 AM - Track 3

CPS Oral Presentations

Hyperpolarizing Gabaergic Transmission Requires the Kcc2 C- Terminal Iso Domain

Acton BA, Woodin MA (University of Toronto)

KCC2 is the neuron-specific member of the of K^+-Cl^- cotransporter gene family. It is also the only member of its family that is active under physiologically normal conditions, in the absence of osmotic stress. By extruding Cl^- from the neuron under isotonic conditions, this transporter maintains a low concentration of neuronal Cl^-, which is essential for fast inhibitory synaptic transmission by GABA and glycine in the mature nervous system. The other members of this K^+-Cl^- cotransporter gene family are exclusively swelling-activated. Here we demonstrate that a 15 amino acid region near the end of the C-terminus, unique to KCC2 (termed the ISO domain), is required for KCC2 to cotransport K^+ and Cl^- out of the neuron under isotonic conditions. We made this discovery by overexpressing chimeric KCC2-KCC4 cDNA constructs in cultured hippocampal neurons prepared from Sprague Dawley rat embryos and assaying neuronal Cl^- through gramicidin perforated patch clamp recordings. We found that when neurons had been transfected with a chimeric KCC2 that lacked the unique ISO domain, hyperpolarizing responses to GABA were abolished. This finding indicates that the ISO domain is required for neuronal Cl^- regulation. Furthermore, we discovered that when KCC2 lacks the ISO domain, it still retains swelling-activated transport, which demonstrates that there are exclusive molecular determinants of isotonic and swelling-induced K^+-Cl^- cotransport in neurons.

Importance of the Mevalonate Pathway in the Development and Survival of Purkinje Cells

Barszczyk A, Smith A, Charlton M, Feng Z-P, Department of Physiology, University of Toronto, Toronto, Ontario, Canada.

Background/Objectives: The mevalonate pathway culminates in the synthesis of cholesterol. The rate-limiting step of this pathway is catalyzed by HMG-CoA reductase, which can be inhibited by statins. Statins are also detrimental to neuronal development and survival; this may be due to the loss of cholesterol or the loss of other products of the pathway. We tested the hypothesis that products of the squalene-dependent, cholesterol-synthesizing branch of the pathway and the cholesterol-independent geranylgeranyl pyrophosphate (GGPP) branch of the pathway have different effects on neuronal development and survival.
Methods: Neuronal development was evaluated by measuring neurite outgrowth and assessing differentiation state, while survival was assessed visually. To identify the components of the mevalonate pathway that are required for either neuronal development or survival, mevastatin was used to inhibit HMG-CoA reductase, and zaragozic acid was used to inhibit squalene synthase. GGPP was added to rescue mevastatin-induced effects on survival.
Results: GGPP alone was sufficient to prevent mevastatin-induced cell death. This occurred in both immature and differentiated Purkinje cells; however, these cells still failed to grow and differentiate. Zaragozic acid was sufficient to inhibit neurite outgrowth and differentiation in cells not treated with mevastatin.
Conclusions: Neuronal development and survival require distinct products of the mevalonate pathway**.**

Glutamate and GABA/Glycine Co-released in the Immature Inhibitory MNTB-LSO Circuit Show Differential Dependence on Calcium

Alamilla, Javier; Gillespie, Deda C (McMaster University)

Background/objectives: The lateral superior olive (LSO) of auditory brainstem is a model system for studies of how immature excitatory and inhibitory circuits are coordinately refined. The immature GABA- and glycin-ergic terminals from the medial nucleus of the trapezoid body (MNTB) also release glutamate, which may be required for early circuit refinement. To understand release of these distinctly different neurotransmitters, we asked whether they are differentially released upon calcium influx.
Results: For GABA/glycine, release probabilities in slices younger than postnatal day 5 (P5) differed from those > P7 in normal to high external Ca^{++} concentrations. For glutamate, no significant developmental trends were found. Surprisingly, L-type calcium channels contributed to release before P5, and N-type channels barely or not at all. After P8, as has been reported at other synapses in the auditory brainstem, P/Q-type calcium channels predominated.
Methods: Recordings from principal neurons of the LSO in response to electrical stimulation in the MNTB in acute slice. Using whole-cell voltage clamp to isolate GABA/glycine and glutamate neurotransmission, we measured response amplitude and paired-pulse ratio with various external calcium concentrations (0.1, 2, and 4 mM) and calcium channel antagonists (nitrendipine, ω-agatoxin IVA, ω-conotoxin GVIA, Cd), as a function of age (P1-15).
Conclusions: Our results are consistent with a model in which GABA/glycine and glutamate are packaged in distinct, physically segregated, vesicle populations within the MNTB terminal.

Hydrogen Bonds are Critical for the Docking and Formation of Funxtional CX26/CX32 GAP Junction Channels

Bai, D.[1]; Gong, X.Q.*[1]; Nakagawa, S.*[2] and Tsukihara, T.*[2] (University of Western Ontario and Inst. Protein Res., Osaka U., Osaka)

Background Gap junctions (GJs) are formed by docking of two hemichannels from adjacent cells. Each hemichannel is a hexamer of connexins. It is known for more than 10 years that docking and formation of functional GJ channels are only possible among compatible connexins, but what 'staple' the two hemichannels together at the docking interface is not clear.
Results Our Cx26 GJ crystal structure model and homology structural model for Cx26/Cx32 GJ channel indicate that there are 60 putative hydrogen bonds (HBs) between each pair interdocked hemichannels (10 HBs from each pair interdocked Cxs, 4 at E1-E1 and 6 at E2-E2). Experimental evidence and sequence alignment analysis on the E1 and E2 domains indicates that E2 domain is more critical for the docking compatibility. We strategically designed point mutations on the HB-forming residues of the E2 of Cx26 and Cx32 to reduce the number of HBs at the docking interface. Our results indicate that reduce the HBs from 6 to 4 (or 5) at the E2-E2 docking interface of homo- or heterotypic GJ channels showed no apparent defect in the coupling conductance of cell pairs expressing these mutants. But, further reduction of the HBs from 6 to 3 or lower at each pair of E2-E2 docking interface, the coupling conductance were virtually eliminated, indicating unlikely to form functional GJ channels. The Cx mutants in this category (including a human disease-linked mutation) can be rescued their GJ function by cells expressing another Cx mutant aiming to re-establish 4 or more HBs between E2-E2.
Conclusion Our study indicates that a minimum of 4 HBs at each pair of E2-E2 of two hemichannels are required to form a functional GJ channel in Cx26 and Cx32.

Intracelluar Trafficking Of Regulator Of G-Protein Signaling 4 (Rgs4) Requires Palmitoylation And Rab Family Function

*Bastin Guillaume, G.B[1,2], and Heximer Scott, S.H[1,3]. [1]Department of Physiology, University of Toronto, Canada, [2]Université des Sciences et Technologies de Lille, France, [3]Heart and Stroke Richard Lewar Centre for Excellence in Cardiovascular Research.

Background/Objectives: RGS4 is a potent protector of many diseases of importance. Indeed, RGS4 was shown to protect us against neurodegenerative diseases, cancers, diabetes and cardio-vascular pathologies.
RGS4 has been localized to both plasma membrane and intracellular membrane pools, however, the nature of intracellular trafficking remains to be been elucidated. G-protein inhibition requires the presence of RGS4 at the plasma membrane. Therefore, using mammalian cells, we hoped linking cellular model to RGS4 physiological function for identifying drug targets.
Results/Methods: The Rab family of proteins is known to facilitate intracellular trafficking of proteins through various vesicular and endosomal compartments via both retrograde and anterograde pathways. We used live cell imaging confocal microscopy to uncover a marked colocalization between RGS4 and Rab11 on intracellular endosomes. We asked whether Rab11, a key element in endosomal protein recycling and exocytosis may be important for plasma membrane targeting of RGS4. Indeed, when co-expressed dominant negative Rab11, RGS4 showed reduced plasma membrane targeting and an impaired ability to inhibit M1 muscarinic receptor mediated signaling. In parallel, we showed that RGS4 plasma membrane levels could also be regulated at the level of the endocytic machinery. Specifically, overexpression of Rab5a, the Rab family member required for clathrin-mediated endocytosis, decreased the presence of RGS4 at the plasma membrane and impaired its M1 muscarinic receptor inhibitory function. Interestingly, addition of 2-BP, an inhibitor of palmitoylation, prevented targeting of RGS4 to endosome compartments and the plasma membrane. Mutations of RGS4 Cysteine2 and Cysteine12, two putative palmitoylation sites, respectively disrupted localization to endosomal structures and plasma membrane targeting, these mutations lowered RGS4 inhibition of Gq mediated signaling.
Conclusions: Taken together, these data provide the first evidence of Rab-dependent intracellular trafficking of RGS4 with the participation of its N-terminal palmitoylation sites and provides the tools for identifying new strategies aimed at increasing the function of RGS4 in living cells.

Thursday 10:30 AM - Track 1

Challenging Real Patient Pharmacology/Toxicology Cases Presenting at Canadian Hospitals

[Abstracts and bios not available]

Thursday 10:30 AM - Track 2

Nanotechnology in Cancer Therapeutics: From Detection to Eradication

SPONSORED BY: PURDUE PHARMA

Nanoparticle Delivery of siRNA and Chemotherapy to Tumors

Leaf Huang, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC

We have developed two different types of membrane/core nanoparticles for delivery of siRNA and chemo drugs. Both contain a PEG brush on the surface lipid bilayer such that little or no nanoparticle uptake by the reticuloendothelial system (mainly the macrophages in liver and spleen) occurs after intravenous administration. The lipid bilayer membrane is supported and stabilized by charge-charge interaction with the solid core which is at the center of the nanoparticle. LPD nanoparticles contain a protamine/DNA/siRNA core and LCP nanoparticles contain a calcium phosphate amorphous precipitate core. Both can encapsulate siRNA and/or a chemo drug (doxorubicin and gemcitabine) with high efficiency. LCP has an added advantage of being acid sensitive to efficiently release the cargo from the endosome into the cytoplasm. Delivery of therapeutic siRNA and/or a chemo drug to human lung cancer xenograft model has been accomplished which leads to tumor apoptosis and growth retardation. Work supported by NIH grants CA129835, CA149363, CA151652 and CA151455.

Leaf Huang
Dr. Huang is the Fred Eshelman Distinguished Professor, Division of Molecular Pharmaceutics in the Eshelman School of Pharmacy, University of North Carolina at Chapel Hill. Dr. Huang's research has been in the area of gene therapy and targeted drug delivery. He has pioneered the liposome non-viral vector and has produced the vector for the first non-viral clinical trial in 1992. His current work centers on nanoparticle vectors for gene transfer in tumor and liver. He also continues research in establishing a ligand targeted delivery system for cDNA, mRNA, siRNA, proteins and peptides for tumor growth inhibition and for vaccines in treating cancer and infected diseases. He has authored or co-authored more than 320 peer-reviewed papers and more than 130 reviews and book chapters. The H-index of his publications is 76. He is also the inventor or co-inventor of 16 US and foreign patents. In 2004, he received the Alec D. Bangham MD FRS Achievement Award, which is highest honor in the field of liposome research. Dr. Huang has also co-founded 5 biotech start-ups in the past.

Drug Delivery with Mitochondria-Penetrating Peptides

Shana Kelley, University of Toronto, Toronto, ON

Eukaryotic cells are complex structures with specialized compartments - organelles - that play important functional roles. We have developed a class of peptide-based conjugates displaying organellar targeting within human cells that can be used to probe specific compartments and deliver agents with chemical or biological activity. The use of these targeting vectors for the delivery of anticancer agents or antimicrobials will be discussed.

Shana Kelley
Shana Kelley is a Professor of Biochemistry and Pharmaceutical Sciences at the University of Toronto. She is an expert in the development of new nanomaterials for biological and cellular sensing –

an area of significant importance for the development of new medical diagnostic technologies. Dr. Kelley obtained her Ph.D. at the California Institute of Technology in Chemistry in 1999. Dr. Kelley has been recognized for her contributions with several awards. She was named one of "Canada's Top 40 under 40" in 2008 and a "Top 100 Innovator" by MIT's Technology Review. This year she was awarded the Steacie Prize, an honour that goes to a Canadian scientist or engineer each year to recognize research achievement.

Lipid Nanoparticle Formulations of siRNA for Gene Silencing in the Liver

Pieter R. Cullis, Department of Biochemistry and Molecular Biology, University of BC

RNAi-based drugs such as siRNA require sophisticated delivery systems in order to achieve therapeutic benefits. These delivery systems must protect encapsulated siRNA from degradation in the circulation, promote accumulation in target tissue and facilitate intracellular delivery into target cells. In order to be suitable for clinical use, these delivery systems must also be relatively non-toxic and must encapsulate siRNA efficiently into well-defined, reproducible nanomedicines using a scalable manufacturing process. Lipid nanoparticles (LNP) are currently the leading delivery systems for satisfying these demands. Efficient loading into LNP can be achieved using ionizable cationic lipids that are relatively non-toxic and can be optimized to achieve maximum intracellular delivery of siRNA following uptake into target cells. With regard to manufacture of LNP siRNA systems, formulation processes require rapid mixing of an aqueous stream, containing siRNA, with an enthanolic solution containing cationic lipid and PEG-lipid. We have devised scalable microfluidic mixing technology that results in the formation of LNP siRNA systems over the size range 20-100 nm with siRNA encapsulation efficiencies approaching 100%. It is shown that LNP siRNA systems containing optimized ionizable cationic lipids are highly potent and relatively non-toxic agents for silencing hepatocyte target genes following i.v. injection, achieving 50% or greater target gene silencing of 10 μg siRNA/kg body weight with therapeutic indices of 1000 or higher. This is currently the world-leading "gold standard" for the potency of siRNA-based therapeutics in vivo.

Pieter R. Cullis

Pieter R. Cullis, Ph.D. Professor, Department of Biochemistry and Molecular Biology, University of British Columbia, Director, NanoMedicines Research Group, UBC. Dr. Cullis and co-workers have been responsible for fundamental advances in the generation, loading and targeting of liposomal nanoparticulate (LNP) systems for intravenous delivery of conventional drugs and genetic drugs such as siRNA. This work has contributed to two LNP products that have been approved by regulatory agencies in the U.S. and Europe for the treatment of cancer and its complications, another that is currently under consideration by the US FDA for the treatment of leukemia and five more that have completed Phase I studies. Dr. Cullis co-founded The Canadian Liposome Company, Inex Pharmaceuticals (now Tekmira Pharmaceuticals), Northern Lipids Inc., AlCana Technologies and Precision NanoSystems. In addition, he co-founded and was Scientific Director (2004-2010) of the Centre for Drug Research and Development (CDRD). CDRD is a national not-for-profit research centre that transforms early stage discoveries in the life sciences into viable investment opportunities for the private sector. He has published over 290 scientific articles and is an inventor on over 40 patents. Dr. Cullis was elected a Fellow of the Royal Society of Canada in 2004 and has received many other honours, including the B.C. Biotechnology Association award for Innovation and Achievement in 2002, the Leadership Award of the Canadian Society of Pharmaceutical Sciences in 2010, and the Prix Galien, Canada's premier prize for achievements in pharmaceutical R&D, in 2011.

Nanoscale Delivery of Drug Combinations for Overcoming Multidrug Resistance in Cancer

Xiao Yu (Shirley) Wu, Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada.

Multidrug resistance (MDR) is one of major factors contributing to the failure of cancer chemotherapy. Metastatic cancer cells are normally resistant to conventional chemotherapy and are difficult to eradicate. Thus various drug combinations have been investigated to overcome MDR in cancer including the use of P-glycoprotein inhibitors with anticancer drugs. We have found that co-delivery of

synergistic drug combinations using the same nanocarrier can circumvent MDR in cancer cells much more effectively than delivering them separately. This presentation will discuss the need for and benefits of nanoscale delivery, as compared to macroscopic delivery (i.e., systemic administration) and microscopic delivery of drug combinations.

Xiao Yu (Shirley) Wu

Dr. Wu is a Professor and Director of Advanced Pharmaceutics and Drug Delivery Laboratory, at the Leslie Dan Faculty of Pharmacy, University of Toronto. She received her Ph.D. degree in Chemical Engineering from McMaster University, Canada in 1993, after Master and Bachelor's training in Polymer Science and Engineering in China. Having completed postdoctoral fellowship for over two years in Pharmaceutics and Drug Delivery at the University of Toronto, she joined the faculty in 1994 as Assistant Professor, and later progressed to Full Professor. Dr. Wu's research interests have included nanoparticulate drug combinations for enhanced chemotherapy of multidrug resistant and metastatic cancer, multifunctional nanoparticulate systems for multimodality imaging and drug delivery, nanotechnology and stimulus-responsive polymers-enabled closed-loop insulin delivery, mechanistic studies, mathematical modeling and computer-aided design of modified release dosage forms. Dr. Wu and coworkers have published about 300 papers, book chapters, proceedings and abstracts, and held 15 issued and pending patents. Dr. Wu has been a referee of numerous journals and an editorial board member of three journals. She has served on scientific committees and organizing committees and has delivered numerous invited presentations at international and national conferences, and many organizations in North America, Europe, Asia and Latin America.

Thursday 10:30 AM - Track 3

Neuroscience Symposium

Presynaptic Vesicle Recycling Mechanisms and Information Transfer

Katalin Toth, Associate Professor, Department of Psychiatry and Neurobiology, Laval University, Québec City, QC, Canada

Synaptic vesicle endocytosis is an important step in synaptic vesicle recycling, it helps to maintain reliable neurotransmitter release. Two major pathways of endocytosis co-exist in the same presynaptic terminal: clathrin-dependent endocytosis of single vesicles and bulk endocytosis leading to the formation of large endosomes. In the first pathway vesicle formation from plasma membrane is dependent on the adaptor protein 2 (AP2), whereas the second pathway involves the generation of intermediate endosomes and utilizes the adaptor complex 3 (AP3). Vesicles derived in these two pathways differ in their molecular content and represent distinct vesicular pools. In particular, AP3 recruits the zinc transporter ZnT3 to the vesicular membrane leading to the formation of zinc containing vesicles. We have shown that these two pools have varied release properties.

We investigated the effect of the absence of various key proteins involved in vesicle recycling on the basic properties of synaptic transmission at hippocampal mossy fibre synapses. We used ZnT3 and AP3 knockout mice lacking vesicular zinc and endosomal vesicle formation respectively to explore physiological properties of synapses between mossy fiber boutons and hippocampal CA3 pyramidal cells. In KO animals EPSCs had slower kinetics and were regulated by altered calcium dynamics especially during high frequency neuronal activity. These changes in synaptic transmission altered postsynaptic firing patterns in CA3 pyramidal cells when mossy fibres were stimulated with a complex stimulus pattern. Detailed investigation of electrophysiological properties of synaptic transmission in AP3 KO mice could help us to understand the role of synaptic vesicle heterogeneity inherent to many types of synapses and involved in normal brain functioning.

Katalin Toth
Dr Toth has received her PhD in 1995 at the Eötvös Loránd University in Budapest, Hungary where she studied the light- and electron microscopic features of projecting neurones and interneurons in the hippocampus. She was a postdoctoral fellow at the Institute Pasteur in Paris in the laboratory of Dr Richard Miles where she worked on the structural and functional properties of individual synaptic interactions between connected pairs of neurones. Later, she joined the laboratory of Dr Chris McBain at the NIH where she worked on various aspects of synaptic plasticity at hippocampal mossy fibres. She established her laboratory at Laval University in 2000. Her current research focuses on the organization of the presynaptic vesicle pool and how heterogeneity among various vesicle pools influence information processing the in the hippocampus.

New Molecules Enhancing Axonal Outgrowth and Regeneration

Zhong-Ping Feng, Associate Professor, Department of Physiology, University of Toronto, Toronto, ON, Canada

Adult central neurons of some lower vertebrates and most invertebrates can regenerate following axonal injury more effectively than mammals. Thus, identifying molecules that enable this regeneration may suggest new directions to stimulate axonal growth in the mammalian CNS. *Lymnaea stagnalis*, a freshwater pond snail, is capable of regenerating their injured axons and reforming cell type-specific synapse, both in vivo and in vitro. *L. stagnalis* has served as a successful model for morphological and functional studies in axonal regeneration, however, inadequate transcriptome information of the snail

limits its use in molecular studies. To overcome this limitation, we have conducted a partial neuronal transcriptome sequencing analysis and deduced 7,712 distinct EST sequences. We further created the first gene chips covering ~15,000 of *L. stagnalis* EST sequences. Our microarray analysis identified 67 sequences with more than 2-fold changes following *in vivo* nerve injury. Using gene silencing approach, we identified a number of novel pro-regenerative molecules in snail. Our first study showed a snail molecule promoted the outgrowth capacity of rodent axons. Our findings suggest a promising avenue to identify new mechanisms of promoting intrinsic regenerative properties of mammalian neurons.

Zhong-Ping Feng
Dr Feng is an Associate Professor in the Department of Physiology at the University of Toronto. She received her M.Sc. Degree in Pharmacology at the University of Alberta in the laboratories of Drs. Tessa Gordon and Bill Dryden, and her Ph.D. degree in Neuroscience at the University of Calgary in the laboratory of Dr. Naweed Syed. She obtained her postdoctoral fellowship in the laboratory of Dr. Gerald Zamponi in the Departments of Biophysics and Physiology at the University of Calgary. Since 2003, she has led her laboratory to identify new ion channels and calcium binding proteins involving synapse development and rhythmic activity. She has received numerous personal awards including a CIHR New Investigator Award, a Heart and Stroke Foundation New Investigator Award and a Premier's Research Excellence Award.

Electrical Microstimulation: Modifying Neural Circuits that are Linked to Cognitive Function

Erik Cook, Associate Professor, Department of Physiology, McGill University, Montreal, QC, Canada

Electrical stimulation of the brain has shown promise in the development of novel therapies and neural prosthetics. As a research tool, electrical stimulation applied with small microelectrodes has the benefit of focal activation of specific neural populations. In spite of its widespread use in the clinic and laboratory, we do not fully understand how electrical current passed through a microelectrode interacts with functioning neural circuits. Past behavioral studies have suggested that weak electrical stimulation (referred to as microstimulation) of sensory cortex produces percepts that are similar to those generated by normal sensory stimuli. In contrast, electrophysiological studies have shown that neural activity produced by brief microstimulation is radically different and longer lasting than normal neural responses. For example, neural activity measured in response to microstimulation in both in vitro and in vivo preparations has usually been characterized with a long temporal spread (i.e., a short excitatory response followed by a long period of inhibition). In this presentation, I will highlight the importance of this technique in research and medicine and present experimental results that characterize the corresponding temporal and spatial spread of neural activity in response to electrical brain stimulation.

Erik Cook
Dr Cook is an Associate Professor in the Department of Physiology at McGill University. He trained as an electrical engineer and worked for NASA, specializing in telecommunication systems before earning his Ph.D. in Neuroscience from Baylor College in Houston. His doctoral work focused on the cellular and electrophysiological properties of neurons. His postdoctoral research focused on systems neuroscience and the visual cortex. Since 2004 he has held a Canada Research Chair in the Physiology of Visual Perception and his lab performs behavioral and electrophysiological studies to understand the link between neural activity in cortex and cognitive function.

Treatment of Stroke using PSD-95 Inhibitors

Michael Tymianski, Professor, Departments of Surgery and Physiology, University of Toronto; Senior Scientist, Toronto Western Hospital Research Institute, Toronto, ON, Canada

All attempts at treating strokes by pharmacologically reducing the human brain's vulnerability to ischaemia have failed, leaving stroke as a leading cause of death, disability and massive socioeconomic loss worldwide. Over decades, research has failed to translate over 1,000 experimental treatments from discovery in cells and rodents to use in humans, a scientific crisis that gave rise to the prevailing belief that pharmacological neuroprotection is not feasible or practicable in

higher-order brains. Our laboratory has been investigating various means to effect pharmacological neuroprotection in animals and in humans. This lecture will describe our work, over the past decade, on neuroprotection by PSD95 inhibitors—promising compounds that uncouple postsynaptic density protein PSD95 from neurotoxic signalling pathways. Inhibiting PSD95 reduced the vulnerability of cultured neurons to excitotoxicity, and reduces brain damage in a range of conditions in which excitotoxic signalling is involved, including stroke, traumatic brain injuries, epilepsy, glaucoma and Alzheimer's disease. Most recently, we used higher-order gyrencephalic non-human primates (NHPs), which bear genetic, anatomical and behavioural similarities to humans, and showed that stroke damage can be prevented in NHPs in which a PSD95 inhibitor is administered after stroke onset in clinically relevant models. Our findings established that tissue neuroprotection and improved functional outcome after stroke is unequivocally achievable by administering PSD95 inhibitors to NHPs. Work is currently under way to test this in humans.

Michael Tymianski

Dr Tymianski is the Interim Head, Division of Neurosurgery, Toronto Western Hospital, Medical Director of the Neurovascular Therapeutics Program, University Health Network and the Director of the Lougheed Microsurgical course. He is a Professor of Surgery and Physiology at the University of Toronto, and a Canada Research Chair (Tier 1) in Translational Stroke Research. He treats patients with brain vascular malformations. His research interests are focused on the cellular and molecular mechanisms of ischemic brain damage, and on the natural history of neurovascular disorders. He is currently a neurovascular surgeon at the Toronto Western Hospital, and a Senior Scientist at the Toronto Western Hospital Research Institute.

Thursday PM - Track 1

CSPT Trainee Oral Presentations

HLA-B*1502 and HLA-A*3101 as Genetic Markers for Carbamazepine-induced Hypersensitivity Reactions in Children

Amstutz U, Ross CJD, Castro-Pastrana LI, Rieder MJ, Shear NH, Hayden MR, Carleton BC (University of British Columbia)

The use of the anticonvulsant carbamazepine (CBZ) is limited by the occurrence of hypersensitivity reactions that include drug-induced hypersensitivity syndrome (HSS) and Stevens-Johnson syndrome (SJS). Although rare, HSS and SJS are life-threatening adverse drug reactions with a very high morbidity and mortality. Two genetic variants in the HLA region, HLA-B*1502 and HLA-A*3101, have been associated with CBZ hypersensitivity. However, no study so far has investigated these associations specifically in children. Here, we assessed the association of HLA-A*3101 and HLA-B*1502 with CBZ hypersensitivity in 42 children with CBZ hypersensitivity, and 91 CBZ-tolerant controls. DNA and comprehensive clinical data on the adverse events from all patients were obtained through the Canadian Pharmacogenomics Network for Drug Safety. Genotyping was performed using real-time PCR. A significant association of HLA-A*3101 was observed with CBZ-HSS (OR 26.0, p=0.0008) and maculopapular eruptions (MPE; OR 7.3, p=0.0007), but not for SJS. Conversely, HLA-B*1502 was associated with CBZ-SJS (OR 38.6, p=0.002), but not HSS and MPE. Combined, the two risk variants were strong predictors of all CBZ hypersensitivity reactions (OR 7.6, $p=2.3x10^{-5}$). This study is the first to replicate the association of HLA risk variants with CBZ hypersensitivity in pediatric patients. Our results also provide new insights on the importance of these predictive biomarkers in a multi-ethnic North American population.

Effect of Human Equlibrative Nucleoside Transporter 1 and Ecto-5'nucleotidase (eN) in Adenosine Formation by Astrocytes under Ischemic Conditions

Chu S, Parkinson FE (University of Manitoba)

Background: Under ischemic conditions, levels of adenosine (ADO) increase up to 100-fold in brain. Intracellular or extracellular pathways of ADO formation from neurons or astrocytes could contribute to these rising levels of ADO.
Objectives: The present study examined release of ADO from primary cultures of cortical astrocytes from wild type C57bl6 (wt) or CD73 knock out (KO) mice, under basal or ischemia-like conditions.
Methods: Astrocytes cultured from wt or CD73 KO mice were incubated with 3H-adenine to radiolabel intracellular ATP. Astrocytes were then subjected to glucose deprivation (GD) or oxygen-glucose deprivation (OGD) conditions by treatment with 2-deoxyglucose (10mM) in glucose-free buffer for 30 min (37oC) in a humidified chamber or for 1 hour (37oC) in 95% N2 and 5% CO2. The effects of dipyridamole (DPR; 30 μM), an inhibitor of ENT1 and ENT2, or alpha, beta-methylene ADP (AOPCP; 50 μM), an inhibitor of CD73 on [3H] purine release from astrocytes was tested.
Results: CD73 KO astrocytes produced less ADO under GD and OGD conditions ($p< 0.001$) than wt astrocytes; INO levels did not differ between wt and CD73 KO cells. Under GD and OGD conditions, ADO levels were significantly higher in wt cultures ($P < 0.001$).
Conclusions: Astrocytes produce ADO, but not INO, via an extracellular pathway that requires CD73. These data confirm the role of CD73 in the extracellular pathway contributing to rising levels of ADO formation under ischemia like conditions.

The Effect of N-acetylcysteine on the Antitumour Efficacy of Ifosfamide in a Mouse Xenograft Model

Hanly LN, Figueredo R, Rieder MJ, Koropatnick J, Koren G (University of Western Ontario)

Background: Nephrotoxicity is a serious side effect, affecting 30% of children treated with the drug ifosfamide. N-acetylcysteine, currently used in children for acetaminophen overdose, has mitigated this renal toxicity in cell and rodent models. Before this treatment can be realized clinically, we must show it does not affect the antitumour efficacy of ifosfamide.
Methods: In a Ewing's sarcoma xenograft mouse model we compared the efficacy of ifosfamide with and without n-acetylcysteine. Ewing's sarcoma tumours were implanted into mice, which were then randomized to receive one of the following treatments: 1) Saline 2) ifosfamide 3) ifosfamide + concurrent n-acetylcysteine 4) pretreatment with n-acetylcysteine + ifosfamide 5) n-acetylcysteine. Tumour volumes were assessed 3 times/week by caliper.
Results: Median tumour volumes of control mice (n=6) were significantly different from median tumour volumes in mice treated with ifosfamide alone (n=8), concurrent NAC, and pretreatment NAC ($p<0.05$). Moreover, when compared to median tumour volumes of mice treated with ifosfamide alone, those treated concurrent and pretreatment NAC, showed no significant difference in tumour growth, and tended to have lower tumour volumes.
Conclusion: These data show no evidence that NAC might interfere with the antitumour efficacy of ifosfamide. They further support to need for a clinical trial to assess the effectiveness of NAC to protect against ifosfamide-induced nephrotoxicity in children.

Investigation of the Cytotoxic Effects of Novel Jadomycins in Drug Sensitive and Drug-resistant Breast Cancer Cells

Issa M, Dupuis SN, Jakeman DL, Goralski KB (Dalhousie University)

Background: Multidrug resistance (MDR) remains a major obstacle in the treatment of metastatic breast cancer. Novel anticancer agents that are efficacious in resistant tumours are needed. Jadomycins are polyketide-derived natural products produced by the soil bacteria *Streptomyces Venezuelae* (ISP 5230). We hypothesise that some jadomycins analogues evade efflux by ABC transporters, and as a result those analogues will exhibit higher cellular accumulation and improved efficacy over existing anticancer agents in drug-resistant tumour cells.
Methods: The cytotoxicity of jadomycin analogues was determined using MTT assays in drug-sensitive (control) and drug-resistant (ABCB1, ABCC1 or ABCG2-overexpressing) MCF7 breast cancer cells. The cellular efflux of the ABCB1, ABCC1 and ABCG2 substrates, respectfully, rhodamine 123, doxorubicin and Hoechst 33432 in stably transfected HEK cells with or without pharmacologically active concentrations of jadomycins was used to determine which jadomycins inhibit these drug efflux transporters.
Results: In comparison to control MCF7 cells, jadomycins G, DNV, B and N were equally toxic to ABCB1-overexpressing MCF7 cells; jadomycins DNV, SPhG and N were equally toxic ABCC1-overexpressing MCF7 cells; and only jadomycin N was equally toxic to ABCG2-overexpressing MCF7 cells. None of the jadomycin analogues inhibited the efflux of ABCB1, ABCC1 or ABCG2 probe substrates in transport assays.
Conclusion: The ability of jadomycins to retain cytotoxic activity in the corresponding drug resistant MCF7 cells stems from their ability to circumvent interactions with the ABCB1, ABCC1 and ABCG2 drug efflux transporters. Based on the favorable pharmacokinetic properties we are further exploring the mechanisms of action and chemotherapeutic potential of these jadomycins.

Ethyl Glucuronide Crosses the Human Placenta and Represents Maternal and Fetal Exposure to Alcohol

Matlow J, Lubetsky A, Aleksa K, Koren G (Hospital for Sick Children)

Background: Alcohol consumption during pregnancy can lead to Fetal Alcohol Spectrum Disorder, and because maternal self-reports are often unreliable, a biomarker of alcohol use during pregnancy is needed to accurately determine fetal exposure. Ethyl glucuronide (EtG) is a direct metabolite of ethanol that has been detected in the meconium of infants born to mothers who consumed

alcohol during pregnancy.
Objective: To determine to what extent EtG crosses the human placenta.
Methods: Placentae (n=4) from consenting women undergoing elective Caesarian section at St. Michael's Hospital in Toronto were taken to the on-site perfusion laboratory. After cannulation and establishment of dual circulation, 1 mcg/mL EtG was added to the maternal reservoir and samples were taken throughout the 3h experiment. Measurements of placental viability were oxygen transfer, pH, glucose consumption, hCG production, fetal reservoir volume, and fetal arterial inflow pressure. EtG was analyzed in perfusate samples and placental tissue by GC-MS after solid phase extraction.
Results: After 3h, the fetal-to-maternal ratio was 0.30 ± 0.02 and net maternal-to-fetal transfer was still occurring. Triplicate averages of EtG concentrations in perfused placental lobules ranged from 140-414 ng/g. Placental validation markers were within normal ranges for all perfusions.
Conclusions: EtG appears to cross the human placenta and, hence, to represent both maternal and fetal exposure to alcohol.

Embryonic Catalase Protection Against Ethanol Embryopathies in Acatalasemic and Human Catalase-expressing Mice in Embryo Culture

Miller L, Wells PG (University of Toronto)

Alcohol (ethanol, EtOH) consumption during pregnancy can cause a spectrum of structural, cognitive and behavioural anomalies termed the Fetal Alcohol Spectrum Disorder (FASD). Reactive oxygen species (ROS**)** have been implicated in the teratogenic mechanism, but the protective role of the embryonic antioxidative enzyme catalase is unclear, as embryonic activity is less than 5% of maternal levels. We addressed this question in a whole embryo culture model. C57BL/6 (C57) mouse embryos expressing human catalase (hCat) or their wild-type (C57 WT) controls, and C3Ga.Cg-*Cat*^{b}/J catalase-deficient, acatalasemic (aCat) mouse embryos or their wild-type C3HeB/FeJ (C3H WT) controls, were explanted on gestational day (GD) 9 (plug = GD 1), exposed for 24 hr to 2 or 4 mg/mL EtOH or vehicle, and evaluated for functional and morphological changes. hCat and C57 WT vehicle-exposed embryos developed normally, while EtOH was embryopathic in C57 WT embryos, evidenced by decreases in anterior neuropore closure, somites developed, turning and head length, whereas hCat embryos were protected ($p < 0.001$). Maternal pretreatment of C57 WT dams with 50 kU/kg PEG-catalase **(**PEG-Cat**)** 8 hr prior to embryo culture, which increases embryonic catalase activity, blocked all EtOH embryopathies ($p < 0.001$). Vehicle-exposed aCat mouse embryos had lower yolk sac diameters compared to C3H WT controls, suggesting endogenous ROS are embryopathic. EtOH was more embryopathic in aCat embryos than WT controls, evidenced by reduced head length and somite development ($p < 0.01$), and trends for reduced anterior neuropore closure, turning and crown-rump length in aCat embryos. Maternal pretreatment of aCat dams with PEG-Cat blocked all EtOH embryopathies ($p < 0.05$). These data suggest that embryonic catalase is a determinant of FASD risk, and that ROS contribute to the embryopathic mechanism.

Lysophosphatidicacid (LPA)-induced Enhancement of Blood-Brain Barrier (BBB) Permeability as a Potential Method for Enhancing Drug Delivery to the Brain

On NH, Miller DW (University of Manitoba)

Background: 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 and may have potential CNS drug delivery applications.
Objectives: Characterize the extent of LPA-induced modulation of BBB permeability and provide initial proof-of-concept for use of LPA to enhance drug delivery to the brain.
Methods: Alterations in BBB permeability were characterized in Balb/C mice using a small (gadolinium contrast-enhanced agent (Gad)), and large (IRdye800cw PEG) vascular permeability imaging agent. In addition, Rhodamine 800 (R800) imaging agent was used to monitor changes in P-glycoprotein-mediated BBB permeability. Mice were also administered 3H-methotrexate, either alone or in the presence of LPA to determine improvements in brain delivery of chemotherapeutic agent.
Results: The magnitude of BBB disruption was greatest for Gad with increases of 20-fold. Macromolecule marker, IRdye 800cw PEG, showed

approximately 3-fold enhancement in brain accumulation following LPA. Increased brain penetration of R800 was observed following LPA exposure. The brain accumulation of methotrexate was increased 17-fold in LPA treated mice compared to vehicle.

Conclusions: LPA produced a rapid and reversible increase in BBB permeability to a wide variety of agents. Use of LPA in combination with therapeutic agents may be an effective strategy to increase drug delivery to the brain.

Ligand-dependent and Receptor-selective Effect of Non-nucleoside Reverese Transcriptase Inhibitors on the Activity of human Pregnane x Receptor and Constitutive Androstane Receptor

Sharma D, Chang TKH (University of British Columbia)

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are used routinely along with other anti-HIV drugs for treating HIV-1 infection. However, when used in combination, certain NNRTIs are known to cause drug interactions. *In vitro* studies have shown that NNRTIs increase mRNA levels of drug-metabolizing enzymes and transporters, the expression of which is regulated by nuclear receptors. However, the mechanism involved in NNRTI-mediated induction of drug-metabolizing enzymes and transporters is not understood. This study was aimed to investigate the interaction between selected NNRTIs and nuclear receptors, such as pregnane x receptor (PXR) and constitutive androstane receptor (CAR). Cytotoxicity of NNRTIs was determined by the lactate dehydrogenase (LDH) assay. The effect of NNRTIs on the activity of human PXR (hPXR) and human CAR (hCAR) (and its spliced variants) was assessed in transiently transfected HepG2 cells. Reporter gene expression was quantified using the Dual-Luciferase Reporter Assay System. Each of the selected NNRTI at concentrations up to 5 μM did not increase LDH release. Efavirenz (7.3-fold), etravirine (17.1-fold), and rilpivirine (11.4-fold), but not nevirapine or delavirdine, activated hPXR. None of the NNRTIs activated hCAR or its spliced variants, regardless of whether or not cells were co-transfected with retinoid x receptor α. Our results indicate a ligand-dependent and receptor-selective effect of NNRTIs on the activity of hPXR and hCAR.

Thursday 4:00 PM - Track 1

CSPT Distinguished Service and Education Award Lecture

The Challenges of Pharmacology in the New Millennium

Patrick du Souich, Professor and Director, Department of Pharmacology, Faculty of Medicine, Université du Montreal

In recent years, we have witnessed extraordinary changes in the discipline of pharmacology, primarily in the area of research. However, these encouraging changes in the discipline fade the identity of pharmacology. The identity of the discipline is closely tied to the teaching and research of pharmacology and therapeutics, and both activities have evolved over the years. The need of a competitive and successful research has favoured the recruitment of experts in specific domains of research, and that independently of the needs for optimal teaching. As a consequence, teaching of pharmacology and therapeutics is not done by experts, as corroborated by the incidence of prescription errors, avoidable drug-drug interactions, and severe ADEs.The research has become progressively organ/system-pathology oriented, e.g. neurosciences, cardiovascular, etc, and less focused on pharmacology. The interest for drug response has shifted to mechanism- and target-oriented, with diminished pharmacological identity. The future of the discipline of pharmacology is associated with the survival of the departments of pharmacology, and that depends on the ability to teach pharmacology and therapeutics to undergraduates
and postgraduates in the medical school or in the frame of continuous medical education, and to perform research with a therapeutic connotation. Optimal teaching and relevant research requires the close collaboration of basic and clinical pharmacologists

Patrick du Souich

Dr. Patrick du Souich obtained a M.D. degree in 1968 from the University of Barcelona, passed the Spanish Boards of Internal Medicine in 1972, and completed a Ph.D. in Pharmacology in 1976 from the University Autónoma of Barcelona, Spain. He did post-doctoral studies in Clinical Pharmacology in San Antonio, Texas (1976-1977) and in Pharmaceutical Sciences in Buffalo, New York (1977-1978) with an International Merck Sharp & Dohme Fellowship. He passed the License of the Medical Council of Canada in 1979 and Boards of Clinical Pharmacology in 1983 (Spain).

Dr. du Souich joined the Department of Pharmacology of the University of Montréal in 1978 where now he is full professor and chairman of the Department. Simultaneously, he has been an active member of Hôpital Hôtel-Dieu de Montréal. His research focuses on the effect of disease on the kinetics, dynamics and biotransformation of drugs. He has published around 250 manuscripts, including 30 book chapters.

He received the K.M. Pfiasky Young Investigator Award in 1983. He was awarded with the Chair Serono of Pharmacology from the Universidad Autónoma de Madrid, Spain in 2000, and he is the recipient of the Chaire Merck Canada en pharmacologie de l'Université de Montréal since 2004.

Dr. du Souich has been member of many institutions and peer review agencies; he has been the President of the Canadian Society for Clinical Pharmacology, 1988-1990. Since 1992, he has occupied various positions in the International Union of Basic and Clinical Pharmacology (IUPHAR), such as Secretary and Chairman of the Clinical Division and actually the President of IUPHAR. He has been Associate Editor of the British Journal of Pharmacology, the Canadian Journal of Physiology and Pharmacology, Therapie and Acta Pharmacologica Sinica. During these years, he has always combined clinical practice, teaching, and fundamental and clinical research.

Thursday PM - Track 2

Advances in Lipid-Based Nanoparticulate Systems for Drug and Vaccine Delivery

SPONSORED BY: TILAK TECHNOLOGIES

Parenteral and Oral Nanodispersions for Small Molecule and Macromolecule Delivery: Biopharmaceutical Considerations and Case Studies

Panayiotis P. Constantinides, Biopharmaceutical & Drug Delivery Consulting, LLC, Gurnee, IL, USA

Lipid-based micro- and nanostructures are widely used in drug discovery, delivery and development. Liposomes and submicron emulsions are used as parenteral vehicles for poorly soluble drugs with several drug products on the market. Also on the market are several oral solutions and liquid-filled capsule lipid formulations that are broadly employed to enhance the solubility and oral bioavailability of BCS II and IV compounds. For more than two decades, however, development of oral formulations of BCS III molecules/macromolecules has been a formidable task but clinical data looks promising. The clinical status of oral peptides using delivery technologies incorporating lipid permeation enhancers will be presented together with other biopharmaceutical considerations of lipid nanodispersions followed by representative case studies. The case studies on the parenteral delivery and targeting of poorly soluble small anticancer drug molecules will focus on nanoemulsions of paclitaxel and liposomal nanocarriers of novel derivatives of non-steroidal anti-inflammatory drugs, such as phospho-ibuprofen (MDC-917). These studies will cover physicochemical characterization and in *vitro/in vivo* antitumor activity using various cancer cell lines and tumor-bearing animals along with a reference to clinical data, if available. In the case of oral lipid nanodispersions, emphasis will be given to reverse micelles and emerging nanoparticles for peptide delivery, such as leuprolide and insulin. The talk will conclude with lessons learned and future perspectives in the field.

Panayiotis (Panos) P. Constantinides
Dr. Constantinides is the Founder and Principal of Biopharmaceutical and Drug Delivery Consulting, LLC, Gurnee, Illinois and Affiliate Associate Professor, Department of Pharmaceutical Sciences, Roosevelt University, College of Pharmacy, Schaumburg, Illinois. He received a University Diploma in Chemistry from Athens University in 1977 and PhD in Biochemistry from Brown University in 1983. He was a postdoctoral fellow in the Pharmacology Department and Associate Research Scientist in the Comprehensive Cancer Center of Yale University School of Medicine (1983-1987). Past industrial positions held included: Vice President of R&D with DOR BioPharma and Morton Grove Pharmaceuticals (2001-2004), Director of Research at SONUS Pharmaceuticals (1997-2000) and from 1987 to 1997 a number of R&D positions of increasing responsibilities with LipoGen, SmithKline Beecham Pharmaceuticals and Abbott Laboratories. Dr. Constantinides has held adjunct faculty positions with the Universities of Tennessee and Washington, he is affiliated with the University of Wisconsin, Extension Services in Pharmacy and serves on the faculty of the Biotechnology Entrepreneurship Boot Camp at the annual BIO International Convention. He is inventor in 30 patents and patent applications and has authored more than 100 technical articles, book chapters, abstracts and conference proceedings. He has been invited speaker at many national and international conferences, pharmaceutical companies and universities. Dr. Constantinides is AAPS Fellow and Past Chair of the AAPS Lipid-Based Drug

Delivery Systems & Nanotechnology Focus Groups.

Multi-Compartmental Lipid Delivery Systems for Cancer Vaccination

Mansoor M. Amiji, Distinguished Professor and Chairman, Department of Pharmaceutical Sciences, School of Pharmacy, Northeastern University, Boston, MA, USA

Despite progress on several preventive and therapeutic fronts, cancer remains one of the major causes of morbidity and mortality worldwide. Development of prophylactic and therapeutic vaccines is considered a novel approach for prevention and treatment of cancer and can work in concert with other approaches including surgery, radiation, and chemotherapy. An ideal cancer vaccine should elicit potent humoral and cytotoxic anti-tumor immune response without any contributing side-effects. As such, the major goal is to stimulate both innate and adaptive immunity that can recognize and subsequently eliminate the tumors mass.

Vaccine delivery systems are generally nano- and micro-particulate systems, such as liposomes, oil-in-water emulsions, and polymeric micro- and nano-particles that can encapsulate the antigen payloads and deliver these to the target site and possibly antigen presenting cells (APC's) – either systemically or upon mucosal administration. In order to effectively targeted APC's for delivery of diverse payloads including peptide, protein, exosomes, and nucleic acid constructs to stimulate immunity, we have developed multi-compartmental delivery systems. Examples of multi-compartmental delivery systems include water-in-oil-in-water multiple emulsions (ME) and nanoparticles-in-emulsion (NiE) formulations. Using gp-100 peptide melanoma antigen or plasmid DNA expressing gp-100 protein, we have evaluated systemic vaccine efficacy using squalane oil-containing ME and NiE formulations in prophylactic and therapeutic immunization protocols in B16 murine melanoma model.

Based on the preliminary results, multi-compartmental delivery systems offer a unique opportunity for encapsulating variety of different antigenic payloads and targeted delivery to APC's upon systemic administration.

Mansoor M. Amiji

Dr. Amiji is currently the Distinguished Professor and Chairman of the Department of Pharmaceutical Sciences and Co-Director of Northeastern University Nanomedicine Education and Research Consortium (NERC) at Northeastern University in Boston, MA. NERC oversees a doctoral training grant in Nanomedicine Science and Technology that was co-funded by the NIH and NSF. Dr. Amiji received the Bachelor's degree in Pharmacy from Northeastern University in 1988 and the Doctorate in Pharmaceutical Sciences from Purdue University in 1992.

His research is focused on development of biocompatible materials from natural and synthetic polymers, target-specific drug and gene delivery systems for cancer and infectious diseases, and nanotechnology applications for medical diagnosis, imaging, and therapy. His research has received over $15 million in sustained funding from the National Institutes of Health (NIH), National Science Foundation (NSF), private foundations, and corporations.

Dr. Amiji teaches in the professional pharmacy program and in the graduate programs of Pharmaceutical Science, Biotechnology, and Nanomedicine. He has supervised research efforts of over 70 post-doctoral associates, both PhD and MS graduate students, and undergraduate honors students over his career. He has published four books and over 200 book chapters, peer-reviewed articles, and conference proceedings.

He has received a number of awards including the NSTI Award for Outstanding Contributions towards the Advancement of Nanotechnology, Microtechnology, and Biotechnology in 2006 and the AAPS Fellowship and AAPS Meritorious Manuscript Award in 2007.

Transcutaneous DNA Immunization: From Nanoparticles to Hair Follicles and Back to Nanoparticles

Zhenrong Cui, Associate Professor of Pharmaceutics, College of Pharmacy, University of Texas at Austin

Transcutaneous immunization onto the skin has become a favorable route for vaccine administration over the traditional use of hypodermic needles and syringes. The feasibility of transcutaneous immunization using plasmid DNA was proven in the 1990's, but the resultant immune responses were generally weak. Several approaches have been taken

to enhance the immune responses from transcutaneous DNA immunization, including physical or chemical disruption of the stratum corneum, the use of vaccine adjuvants or skin permeation enhancers, or formulating the plasmid into carrier systems such as nanoparticles or emulsions. However, all these approaches have had only limited success at enhancing the immune responses. In this presentation, I will summarize the findings from our efforts in the past 10 years to enhance the immune responses induced after transcutaneous DNA immunization by delivering the DNA using nanoparticles, modifying the hair follicle cycles, and facilitating the DNA uptake using microneedle technology.

Zhenrong Cui
Dr. Cui is currently an Associate Professor of Pharmaceutics at the University of Texas at Austin, College of Pharmacy. His research is focused on drug and vaccine delivery. One of his long-standing research interests has been on enhancing the immune responses induced after transcutaneous DNA immunization.

BCS Class IV Compound Case Study: Development and Evaluation of a Novel Oral Amphotericin B Formulation for the Treatment of Systemic Fungal Infections and Drug-Resistant Visceral Leishmaniasis (VL)

Kishor M. Wasan, Faculty of Pharmceutical Sciences, University of British Columbia (NGDI-UBC)

Our laboratory has made significant strides toward the development of a lipid-based amphotericin B formulation for oral administration. Initial data from both cell lines and in vivo research indicate that it is highly efficacious and exhibits low toxicity within the dosage range required in treating diseases such as systemic fungal infections and leishmaniasis. Each year in the Indian subcontinent alone, over 500,000 individuals play host to Leishmania donovani, an insidious parasite that invades macrophages, rapidly infiltrates the vital organs and ultimately leads to severe infection of the visceral reticuloendothelial system. Visceral leishmaniasis, also known as Kala-azar, is most prevalent in the weak and the young within a population. Left untreated, almost all infected individuals will die. The therapeutic arsenal against Leishmania is limited to a small number of parenterally administered agents, with daily injections of pentavalent antimony compound for 28 days being the usual course of action. Due to increasing resistance, antimonial drugs can no longer be used in many areas, including northeastern India where the incidence of Kala-azar is highest. Amphotericin B is the current secondary treatment of choice against leishmaniasis and has a 97% cure rate with no reported resistance. However, therapy with the first-generation formulation (Fungizone®) involves IV administration over a period of 30 to 40 days and is associated with infusion and drug-related side-effects (infection of the indwelling catheter, patient chills and shaking due to RBC haemolysis, dose-dependent renal toxicity, fever, bone pain, thrombophlebitis). Although lipid-based second-generation formulations exist (Abelcet® and AmBisome®), which require a shorter course of therapy (3-5 days), are highly effective and exhibit lower toxicity when compared to Fungizone®, the cost of these formulations is a barrier to widespread use. Due to the difficult route of drug administration, toxicity issues and cost, amphotericin B is failing to reach the infected population and mortality rates continue to rise. The development of an inexpensive, safe and effective oral treatment is paramount in order to address both early and late stages of this deadly disease and drug-resistant forms of VL. This talk will highlight our current findings and future goals.

Kishor M. Wasan
Dr. Kishor M. Wasan is a Distinguished University Scholar Professor, Director and Co-Founder of the Neglected Global Diseases Initiative at the University of British Columbia in Vancouver, BC, Canada. In the 17 years that Dr. Wasan has been an independent researcher at UBC, he has published over 200 peer-reviewed articles and 240 abstracts in the area of lipid-based drug delivery and lipoprotein-drug interactions. His work was recently highlighted in the January 2008 Issue of Nature Reviews, Drug Discovery. Dr. Wasan did his undergraduate degree in Pharmacy at the University of Texas at Austin and his Ph.D. at the University of Texas Medical Center in Houston Texas at MD Anderson Cancer Center in Cellular and Molecular Pharmacology. After completing a postdoctoral fellowship in Cell Biology at the Cleveland Clinic, Dr. Wasan joined the Faculty of Pharmaceutical Sciences at UBC.
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 and on September 30, 2010 Dr. Wasan was named a Fellow of the Canadian Academy of Health Sciences. In May 2011, Dr. Wasan was award the Canadian Society of Pharmaceutical Sciences Leadership award for outstanding contributions to Pharmaceutical Sciences in Canada. Currently Dr. Wasan's research is supported by several grants from The Canadian Institutes of Health Research (CIHR), The Natural Sciences and Engineering Research Council of Canada (NSERC) and several Pharmaceutical companies.

Thursday PM - Track 3

Conducting BE Studies and Other Clinical Trials in Canada and Foreign Countries

Canadian Sponsor Perspectives: Challenges and Opportunities in Outsourcing Bioequivalence Studies

Manon Belisle, Director Biopharmaceutics & Clinical Development, Teva Canada Ltd., Toronto, Canada

Key aspects involved in the CRO selection process by a Canadian Sponsor will be discussed. The discussion will also address the challenges and opportunities of conducting bioequivalence studies in emerging countries. Cost and time saving does sometime come with higher risks; How to mitigate and avoid those risks is paramount for success.

Manon Belisle
Manon Belisle is the Director of the Biopharmaceutics and Clinical Development Department at Teva Canada, a generic pharmaceutical company located in Toronto. In this role, she provides scientific leadership and managerial direction to the team responsible for all activities related to bioequivalence, clinical and toxicology studies. This work leads to generic drug approvals in Canada. Furthermore her team at Teva is also responsible of all study monitoring for studies conducted in Canada by Teva Global. She also held a similar position as Associate Director in Biopharmaceutics with ratiopharm Canada, before the company was acquired by Teva. In the past, Manon has held positions of Senior Pharmacokinetic Scientist and Clinical Manager at MDS Pharma Services, a global CRO located near Montreal. At MDS, she was responsible for developing protocol synopsis and clinical development plans for over 400 bioequivalence for pharmaceutical products from various therapeutic areas. Manon is a graduate of the Université de Montréal and Université du Québec in Montreal. She obtained a Bachelors of Science, Biochemistry in 1987 and a Masters of Science, Physiology in 1991. During 5 years after obtaining her degrees she acted as a researcher for the University of Montreal in fundamental research for different areas including molecular biology, neurophysiology and metabolism. Manon practices mind and body fitness incorporating meditation and physical activities in her life. In her spare time, Manon also loves to spend time in the outdoors appreciating the nature while skiing, canoeing and kayaking. She is an avid hockey player and loves to read, cook and travel. As well, Manon is deeply interested in conscious living, appreciative inquiry and their application in professional and personal spheres.

BE Study Conduct in Canada, Pakistan, Poland, and Egypt: A Canadian CRO Perspective

Lorelei Lutter, Vice President of Business Development, BioPharma Services Inc., Toronto, Canada
Levon Yeghikyan, General Manager, BioPharma Metrics Inc., Karachi, Pakistan

The Bioequivalence (BE) Study regulatory requirements in US, Canada, EU are well defined and well known amongst industry. However, the same cannot be said regarding other jurisdictions. This talk will cover a Canadian CRO's perspective in the BE study experience and requirements in Canada, and compare it with those in other jurisdictions including Eastern Europe, South Asia, and Middle East. It will also cover the challenges faced when conducting BE studies in various countries outside of Canada, including Pakistan, Poland and Egypt. Case studies will be presented throughout the talk to highlight lessons learned, and recommendations to sponsors and the regulators.

Lorelei Lutter

Lorelei Lutter is currently the Vice President of Business Development at Bio Pharma Services Inc., an FDA-inspected, Toronto-based Contract Research Organization (CRO) specializing in Phase I/IIa clinical trials and BE/BA/PK studies in healthy volunteers, special populations, and patient populations for pharmaceutical, biotech and medical device companies globally. She received her Honours Bachelor of Science degree in Human Biology and Nutritional Sciences from the University of Toronto in 1992, and a Master of Business Administration degree from York University, Schulich School of Business, in 2000. She has over 20 years of CRO and pharmaceutical industry experience, in both technical and business development areas. Previous to Bio Pharma, Lorelei held various senior business development, sales and marketing roles in several Canadian CROs including Cantest Ltd. (now Maxxam), Biovail Contract Research (now Lambda), and Pharma Medica Research. She was also involved in managing and outsourcing over 150 BE studies while at Genpharm (now Mylan) in the Scientific Affairs department. Her professional interests are in the areas of global bioequivalence studies, clinical pharmacokinetics, bioanalysis, Phase I/IIa clinical trials, clinical endpoint trials, contract research, international sales and marketing, and outsourcing. She is member of the American Association of Pharmaceutical Scientists (AAPS), and she has been elected as Member-at-Large for the Canadian Society for Pharmaceutical Sciences (CSPS) for 2007-2012, and was appointed to Secretary in 2008-2011.

Levon Yeghikyan

Levon Yeghikyan is General Manager at BioPharma Metrics Inc., a subsidiary of Bio Pharma Services Inc. of Toronto, Canada. He has previously served as Director of Clinic Operations and Subject Recruitment and Screening at BioPharma Services Inc., from 2007-2011, and he has worked at Ventana (now Kendle/INC) and Biovail Contract Research (now Lambda Canada) for many years.

Running Clinical Trials in India: History, Current Environment and Prospects for the Future

Alfred Elvin, Anatase Biopharmaceutic Consulting, New Jersey, USA

Clinical trials for US FDA and EU submission have been conducted in India for over a decade. The first successful FDA ANDA submission using Indian CRO's occurred in 2000. The success generated a rapid increase in the number of CRO's capable of conducting a complete range of clinical and preclinical trials at costs substantially lower than North America. CRO capability, reliable execution and regulatory acceptance have steadily evolved. Regulatory oversight by the India Regulatory Authority (DCGI) has evolved along with the increase in numbers CRO's and trials. Recent changes in DCGI T-license and BE NOC rules have added complexity to study conduct and scheduling. The presentation will address evolution of the India clinical trial environment from its beginning through the current environment and prospects for the future.

Alfred Elvin

Dr. Alfred Elvin has over 28 years of experience in the pharmaceutical industry, including innovator and generic pharmaceutical companies, such as Astra (now Astra Zeneca), Upjohn (now Pfizer), Marrion Merrell Dow (now Sanofi Aventis), Boehringer, Zenith/Ivax Pharmaceuticals (now Teva), Par Pharmaceuticals, and Sandoz. He also has his own consulting firm, Anatase Biopharmaceutic Consulting. Dr. Elvin is one of the pioneers in the conduct of BE studies in India, having started outsourcing there in 2000. He has also served as Chief Technical Advisor for the Traditional Medicine Program of the United Nations Industrial Development Organization, and has outsourced hundreds of BE studies and other clinical trials on a global basis.

Outsourcing or In-house of Conduct of BE Studies in Canada, US and Rest of the World- Create Competitive Advantage

Hari Sankar, Watson Pharmaceuticals, Navi Mumbai, India

Bioequivalence studies are a critical requirement and often determine the success in the launch of generic formulations. The ever reducing profit margins in generic business is a good reason to cut costs and keep this on the priority list of all generic pharmaceutical R&D companies. Generic companies across the globe are trying to enhance the competitive advantage to capture the first to file opportunity to acquire the limited exclusivity in market share. The innovator companies are also adopting new strategies like 'authorized generics' to sustain their market existence after the patent expiry. In this tough and highly competitive scenario, the strategy for performing BE studies becomes crucial and is based upon four important parameters, i.e., Quality, Time, Cost and Confidentiality. The decision of outsourcing BE studies in the US, Canada and in rest of the world is mainly based upon these four parameters. Compromising on any of the four factors can have significant impact on the business. Apart from these four parameters, there are other important factors like regulatory approval status of the CRO, availability of special populations for the conduct of study, expertise in the field of BA/BE and the service level of the CRO mainly with respect to handling the post submission queries. Due to high attrition rate of expertise and cost competition, many CROs are facing challenges to sustain their credibility in the market. In order to meet these challenges, pharmaceutical companies in India as well as many multinational companies are increasingly adopting a strategy of establishing their own in-house BE centres and this has been proven to be an efficient business model in last 8-10 years. Conforming to the regulatory environment and maintaining cost competitiveness in an in-house BE centre is also a challenge. Therefore, It will be interesting to understand the benefits and the risk factors of outsourcing the BE studies in 3rd world/conducting in-house to stay ahead of competitors is crucial for generic business.

Hari Sankar

Dr. Hari Sankar is one of the pioneers in India to initiate BE studies for regulatory submission since 1996. He was instrumental in shaping the early Indian guidelines for BE studies. He has over 15 years experience in BE studies and established successful outsourcing strategies for top companies in India like Dr. Reddy's and Lupin. He is unique for his experience in working on both sides of business ie. sponsor and CRO. He himself is a technical expert to conduct these studies and has an experience of heading CROs like Vimta and GVK, and started a BE centre for Watson. His experience includes obtaining approval for studies conducted from various regulatory agencies of US, Canada, EU, Australia Brazil, South Africa and WHO.

Apart from his clinical experience he is also experienced in manufacturing and R&D operations. A pharmacist and biotechnologist by education. He is versatile in many areas of pharmaceutical operations.

Recommendations for Improving Quality of Bioequivalence Data Submitted to ANDAs in the US

Barbara Davit, CDER, US-FDA, Rockville, MD

[Abstract and bio not available]

Thursday 4:30 PM - Track 1

CPS Distinguished Lectureship Series

JAF Stevenson Visiting Professor Lecture:

Wired for Sound: Establishing Excitatory-inhibitory Balance in Auditory Brainstem

Deda Gillespie, McMaster University

[Abstract and bio not available]

Sarrazin Award Lecture:

Towards Modern Therapeutics for Pulmonary Vascular Disease: Inspiration from new Mechanistic Insights

Duncan Stewart, Ottawa Heart Institute

[Abstract and bio not available]

Friday 8:30 AM - Track 1

Cardiovascular Disease and Global Health

SPONSORED BY: NRC RESEARCH PRESS

Diabetes Therapeutics and Cardiovascular Disease

Daniel Drucker, Mount Sinai Hospital

Type 2 diabetes is associated with increased risk of heart attacks, strokes, and both microvascular and macrovascular disease. Although control of glucose significantly attenuates the development and progression of microvascular disease, the rates of macrovascular disease are not significantly reduced by improvements in glycemic control in studies of medium term 5-10 years duration. This lecture highlights the cardiovascular challenges posed by diabetes and the putative cardiovascular benefits associated with various anti-diabetic agents, with a focus on recently approved incretin-based agents now employed for the treatment of type 2 diabetes.

Daniel Drucker
Dr. Drucker received his M.D. from the University of Toronto in 1980, and was appointed to the Division of Endocrinology, Department of Medicine, University of Toronto in 1987. He is currently Professor of Medicine and holds a Canada Research Chair in Regulatory Peptides and the Banting and Best Diabetes Centre-Novo Nordisk Chair in Incretin Biology. His laboratory is based in the Samuel Lunenfeld Research Institute at Mt. Sinai Hospital in Toronto

New Concepts in the Physiology of Sudden Cardiac Death in the Young

Michael Gollob, Director, University of Ottawa Heart Institute; Associate Professor, Department of Medicine and Department of Cellular and Molecular Medicine, University of Ottawa

Sudden cardiac death in previously well, young athletes is a tragic and often difficult event to comprehend. In previous decades, these tragedies were most commonly attributed to structural diseases of the heart, and in those with normal autopsies, the event remained unexplained. In recent years, genetic studies have identified numerous 'purely electrical' conditions of the heart that typically remain asymptomatic until sudden, deadly arrhythmias occur. Many of these conditions are now understood at the molecular level, although pharmacologic strategies to reduce risk are not yet fully developed. This presentation will highlight some of the recent advances in understanding the pathophysiology of sudden cardiac death in otherwise healthy, young individuals.

Michael Gollob
Dr. Gollob is a native of Toronto, Ontario, and obtained an undergraduate degree in molecular genetics at the University of Toronto, graduating as a Gold Medalist. He then entered the field of medicine and is now a Clinical Electrophysiologist at The University of Ottawa Heart Institute. His clinical and research interests combine his expertise in both genetics and arrhythmia disorders. He is the Director of The Inherited Arrhythmia Clinic and Arrhythmia Research Laboratory at the Ottawa Heart Institute.

Dr. Gollob's research focuses on the genetic and physiological basis of cardiac arrhythmia

syndromes, including sudden death syndromes and the common arrhythmia of atrial fibrillation. He has Chaired on behalf of the CCS the first document outlining the appropriate use of genetic testing for cardiac diseases associated with a risk of sudden death.

His research has led to the identification of the first gene responsible for familial Wolff-Parkinson-White, and 2 novel genes associated with lone atrial fibrillation. He has published his research findings in leading journals, such as The New England Journal of Medicine and Circulation. His laboratory also develops mouse models of human arrhythmogenic disorders. Dr. Gollob is a recipient of the Clinician Scientist Award from the Heart and Stroke Foundation of Ontario and holds peer-reviewed research funding from the Heart and Stroke Foundation and the Canadian Institutes for Health Research (CIHR).

Genetic Predisposition for CAD

Robert Roberts, Ruddy Canadian Cardiovascular Genetics Centre, University of Ottawa Heart Institute, Adjunct Professor of Medicine, Baylor College of Medicine

Susceptibility to coronary artery disease (CAD) is claimed to be 40-60% inherited, but until recently genetic risk factors predisposing to CAD have been elusive. Comprehensive prevention of CAD requires manipulation of genetic risk. The availability of microarrays of single nucleotide polymorphisms enabling genome-wide association studies (GWAS) led to the discovery of 33 genetic risk variants for CAD. Surprisingly, 23 risk variants mediate their risk through unknown mechanisms, with only 10 associating with hypertension or lipids. Thus, there are several mechanisms contributing to the pathogenesis of CAD yet to be elucidated. The first risk variant discovered by GWAS was 9p21.3 which occurs in 75% of all populations except African, with a mean increased risk of 25% per copy. Of the 33 variants for CAD, the increased risk varies from 6-92% with a mean increased risk of 18%, occurring on average in 47% of the population. The maximum number of risk alleles per individual would be 66. In the CARDIoGRAM study of 23 variants, the average per individual was 17, the minimum 7, and the maximum 37. The top 10th percentile has an odds ratio of 1.88 and the lowest percentile an odds ratio of 0.55. Routine genetic screening is unlikely until management is improved by genetic testing. Risk variants should provide patho-physiological insights and targets for novel therapy. While risk variants are less potent predictors of CAD, compared to a biomarkers, they have the advantage of not changing in one's lifetime and are unaffected by diet, gender, age or medication.

Robert Roberts

Dr Roberts received his M.D. from Dalhousie University, completed his residency in Internal Medicine and Fellowship in Cardiology at the University of Toronto. Funded by a Canadian Heart Foundation Scholarship, he pursued research in heart disease at the University of California, San Diego, following which he was recruited to Washington University as Director of the Cardiac Care Unit at Barnes Hospital. In 1982, Dr. Roberts accepted a position as Chief of Cardiology at Baylor College of Medicine in Houston, Texas, where he remained for 23 years. On April 1, 2004, Dr. Roberts was appointed President and CEO of the University of Ottawa Heart Institute and Director of The Ruddy Canadian Cardiovascular Genetics Centre.

Dr. Roberts has had a distinguished and prolific career as a Cardiologist, Educator and Scientist having published over 800 scientific articles and was awarded the **Most Highly Cited Researcher** in 2002. He is a member of the Editorial Board of many journals including being the Editor of Current Opinion in Cardiology. He developed the MCBK Test which has been used to diagnose heart attacks for the past three decades. Dr. Roberts is regarded as one of the founders of molecular cardiology which led him to the discovery of several genes for heart disease.

Dr. Roberts has received many awards including: Distinguished Scientist Award from the American College of Cardiology (1998); Award of Meritorious Achievement from the American Heart Association (2001); McLaughlin Award from the Royal Society of Canada (2008); Albrecht Fleckenstein Memorial Award from the International Academy of Cardiology (2008); Master of the American College of Cardiology (2007); and Robert Beamish Leadership Award (2005).

Cardiometabolic Risk: Role of Genetics

Robert Hegele, Robarts Research Institute, University of Western Ontario

Asking a patient about their family history is a central part of clinical assessment of cardiovascular disease. Thanks to new technology, we may be close to replacing the family history with testing a patient's genetic profile and disease risk. This promises to allow for personalized diagnosis, disease prediction and selection of treatment. But while such potential applications are being actively promoted by genomic scientists and geneticists, how realistic are these promises? Among the ~3 billion DNA bases in a single human genome, ~1% differs 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, lipids and diabetes. 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 method uses leading-edge DNA sequencing to study rare variants and their impact on cardiovascular disease. While proven genetic effects on cardiovascular risk factors are statistically detectable in populations, they are usually modest. In contrast, some very rare mutations in a single gene can greatly increase risk. Understanding pathogenesis in these families may be very important. Some companies are marketing DNA tests directly to consumers, so if only for this reason, it is important for health care providers to understand basic theory, methodology and interpretation of genomic analysis.

Robert Hegele

Dr Hegele is Distinguished University Professor of Medicine and Biochemistry, University of Western Ontario, and director of the Lipid Genetics Clinic and the London Regional Genomics Centre in London, Ontario. He received his MD degree from the University of Toronto in 1981. His specialty training in internal medicine and in endocrinology & metabolism was also in Toronto. His post-doctoral research fellowships were at Rockefeller University and Howard Hughes Medical Institute, University of Utah. From 1989-1997 he was on the Faculty of Medicine at the University of Toronto. In 1997 he joined the Schulich School of Medicine and Dentistry and the Robarts Research Institute at the University of Western Ontario, where he holds the Jacob J. Wolfe Distinguished Medical Research Chair and the Martha Blackburn Chair in Cardiovascular Research. His lab studies the genetics of lipoprotein metabolism, cardiovascular disease and diabetes mellitus. Solely or through collaborations, his lab was first to describe the molecular genetic basis of 12 human diseases. His lab has also defined much of the genetic basis of such complex traits, including hypertriglyceridemia. He has (co-)authored over 460 peer-reviewed publications and has contributed to national treatment guidelines for dyslipidemia, hypertension and diabetes. He is a member of the American Society of Clinical Investigation and the Canadian Academy of Health Sciences, among several other professional organizations. He is associate editor of the *Canadian Journal of Cardiology*, and serves on the editorial boards of the *Journal of Lipid Research* and several American Heart Association journals. He has trained numerous physicians and graduate students.

Friday 8:30 AM - Track 2

Obstacles in Drug Absorption with Emphasis on Disease and Pathophysiological Conditions

Absorption Profile may be Different between Healthy Volunteers and Patients: Obstacles in Drug Development; Do Animal Models Help?

Fakhreddin Jamali, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

A great deal of effort has been expended to predict absorption characteristics of drugs using in vitro and animal data. These predictions, however, generally focus on predicting drug absorption in healthy humans and not the actual patient. The rate and extent of drug absorption, however, may depend on the pathophysiological condition of the patient. This is important during both the R&D and post-marketing phases. During the development phase, generally, the proof-of-concept' is tested in humans and/or in animals models under healthy conditions assuming that improved absorption translates to improved onset of action or effectiveness. However, evidence has been emerging suggestive of significant differences between the healthy and disease conditions with regard to the absorption patterns of various drugs. When slow and/or erratic absorption are observed, attention will often goes to the drug's low aqueous solubility and/or inefficient transport systems and not the disease condition. Indeed, regardless of the formulation used, delayed and erratic absorption patterns have been reported during pain, stoke, Parkinson's disease, arthritis, Behçet's disease, renal impairment, celiac and other gastrointestinal diseases, old age and gluten intolerance. In addition, altered presence of proteins involved in the transport of drugs. For example, at least in vitro and in vivo in rodents, inflammatory conditions result in reduced expression of several transporters such as p-glycoprotein and various multiple drug resistance proteins. Altered expression of these proteins may influence drug bioavailability although human data yet to be reported. Accordingly, attempts to improve physicochemical properties of pharmaceutical products may fail if pathophysiology of the patient is ignored. In addition, bioequivalence during the healthy state does not necessarily translate to the disease condition. The use of appropriate animal models may be helpful during the development phase.

1. Jamali F, Kunz-Dober CM. Pain-mediated altered absorption and metabolism of ibuprofen: an explanation for decreased serum enantiomer concentration after dental surgery. Br J Clin Pharmacol. 1999, 47:391-6.
2. Jamali F, Aghazadeh-Habashi A. Rapidly dissolving formulations for quick absorption during pain episodes: ibuprofen. Int J Clin Pharmacol Ther. 2008, 46:55-63.
3. Nyholm *D,* Lennernäs, H. Irregular gastrointestinal drug absorption in Parkinson's disease. Exp Opin Drug Metab Toxicol. 2008, 4: 193-203.
4. K ChalebyK, el-Yazigi A, Atiyeh M. Decreased drug absorption in a patient with Behcet's syndrome. Clin Chem 1987, 33: 1679-81.
5. Hanafy S, El-Kadi AOS, Jamali F. Effect of Inflammation on Molecular Targets and Drug transporters. J Pharm Pharma Sci 2012, 15 : 361-75.

Fakhreddin Jamali

Dr. Jamali (Doctor of Pharmacy, University of Tehran, Iran; MSc, pharmaceutics, PhD, pharmacokinetics, University of British Columbia, Vancouver, Canada) is a professor at the Faculty of Pharmacy and Pharm. Sci., University of Alberta. He joined the faculty at the University of Alberta in 1981.

His research interests include effect of pathophysiological changes on the action and disposition of drugs, stereochemical aspects of drugs action and disposition, basic and clinical pharmacology of anti-rheumatic, analgesic and cardiovascular drugs, and toxicology of nonsteroidal antiinfammatory drugs. He has published over 200 refereed articles (H-Score, 36), has been an invited speaker at many conferences, and has trained more than 30 PhDs.

Dr. Jamali, a CSPS and AAPS fellow, has received many national and international awards

including the CSPS Leadership Award and the Alberta Centennial medal.

Dr. Jamali has served as a consultant and/or a member of the board of directors of many pharmaceutical houses; He is the founding president of Canadian Soc. Pharm. Sci., editor-in-chief of J. Pharm. & Pharm. Sci., the first open-access journal in the field (www.cspsCanada.org); He teaches pharmacokinetics and clinical pharmacology.

Absorption Profile in Special Populations: Effect of Age

Nikoletta Fotaki, University of Bath, Bath, UK

Development, availability, and use of effective and safe medicines for special age populations are challenging. A better understanding of the effect of age and the related changes in physiology is needed in order to design more appropriate dosage forms for special age groups. Physiological changes and their influence on drug absorption following oral administration to the special age populations will be presented. Furthermore, key factors for the assessment of drug absorption in these populations will be discussed. Implementation of the understanding of how age-dependent physiological factors and practical issues surrounding drug administration influence the absorption and bioavailability of drugs would facilitate the development of oral formulations for special age populations.

Nikoletta Fotaki
Dr Nikoletta Fotaki is a UK registered Greek Pharmacist, with an MSc in Toxicology and a PhD in Biopharmaceutics-Pharmacokinetics. She participated in several research projects in the School of Pharmacy of the National and Kapodistrian University of Athens and in Hoffman La Roche (Pharmaceutical and Analytical R&D, New Jersey, USA) before her academic appointment at the University of Bath (Bath, UK). She has also worked in the National Organisation for Medicines in Greece. Her expertise and research are focused on drug absorption, biorelevant dissolution methods, development of in vitro-in vivo correlations and relations (immediate release and controlled release formulations), formulation development, animal models for the prediction of absorption, methods for reduction/ refinement/replacement of animal experimentation, biowaivers, dissolution testing, design of BA/BE studies, in silico models, integrated software and PBPK modeling. She is a member of several scientific societies and has been an invited speaker at several conference seminars.

Bioavailability Profile as Predicted by Simulation Approaches

Raimar Löbenberg, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Biowaivers are the outcome of applying the scientific knowledge of the BCS to regulatory sciences and product lifecycle management. The talk will review the history and evolution of biowaivers over the past decade. Examples will be given for different BCS classes and how biowaivers can be justified for them. Future directions of biowaivers and the relationship to Quality by Design will be discussed.

Raimar Löbenberg
Dr. Löbenberg holds a BS in pharmacy from the Johannes Gutenberg-University in Mainz, Germany. He received his PhD in pharmaceutics from the Johann Wolfgang Goethe-University in Frankfurt in 1996. He worked on the fundamentals of the Biopharmaceutical Drug Classification System (BCS). He joined the University of Alberta in 2000. His research interests are in dissolution testing to predict the oral performance of dosage forms and in Biopharmaceutics. In recent years, he has consulted different pharmaceutical and nutraceutical companies in their product development and helped them to set product specifications according to the BCS. The application of nanotechnology for drug delivery is another major research interest. Here Dr. Löbenberg investigates the pulmonary delivery of drugloaded nanoparticles to treat diseases like lung cancer.

He is the representative of the Association of Faculties of Pharmacy in Canada to the USP Convention; he is a member of the USP Dietary Supplement Expert Committee and member of the USP Membership Committee. He is Senator of the University of Alberta, member of the AAPS Steering Committee for In Vitro Release and Dissolution; Vice Chair of the Specialty Committee of Traditional Chinese Medicine in Pharmaceutics; World Foundation of Chinese Medicine Science. He is President-Elect of CSPS.

Friday 11:00 AM - Track 1

New Insights in Nephrotoxicity in Children

Drug-Induced Renal Injury: Burden of Illness and Implications for Drug Development and Regulation

Marissa Battistella, University Health Network, Toronto General Hospital, Toronto, Ontario

The kidney receives a blood flow of 25% of resting cardiac output and plays an important role in the elimination of many drugs and their metabolites, thus making it vulnerable to drug toxicity. Drug induced renal injury contributes up to 25% of all cases of acute renal failure. The main mechanisms of nephrotoxicity are vasoconstriction, altered intraglomerular hemodynamics, direct tubular cell toxicity, interstitial nephritis, crystal deposition, thrombotic microangiopathy and osmotic nephrosis. The current standards to monitor renal safety are late, insensitive and show only limited specificity. Recently new kidney safety biomarkers have been developed to detect acute kidney injury in preclinical trials and have proven their superiority in a number of investigational studies in humans. These diagnostic and prognostic biomarkers that are specific for early stages of kidney injury have been provided by metabolomics along with genomics and proteomics technologies. This presentation will review metabolomic approaches for discovering new biomarkers involved in the diagnosis of drug-induced nephrotoxicity and will also discuss the their application in helping to localize and characterize the pathology of the injury.

Marissa Battistella

Dr Battistella graduated from the Faculty of Pharmacy at the University of Toronto in 1998 and completed her pharmacy residency at Sunnybrook and Women's Health Sciences Centre in 1999. She has worked at the University Health Network since 1999 in various positions, including cardiology and internal medicine. In 2002, Marisa completed her Pharm D thru Idaho State University. She has worked as a clinical pharmacist specialist in the hemodialysis unit at the University Health Network since 2002. In the past 10 years, Marisa has published several papers and given many presentations on drug therapy in the area of nephrology. Recently, Marisa has accepted the position of Clinician Scientist jointly between the University Health Network and the Leslie Dan Faculty of Pharmacy where she will focus much of her time on clinical research in the area of nephrology.

Drug-Induced Renal Injury in Special Populations: Neonatology and Oncology

Michael Rieder, Biotherapeutics Research Group, Robart's Research Institute, University of Western Ontario

Drug-induced renal injury is increasingly appreciated as an important clinical problem, notably for chronic diseases whose management is highly dependent on pharmacotherapy. There are many patient populations whose natural history and therapy place them at special risk. In the case of the neonate – notably the premature neonate – developmental ontogeny of renal function places these infants at special risk. This is germane in that many of the most commonly used drugs – for example, the aminoglycoside antibiotics – are both highly dependent on renal excretion and have significant potential for renal injury in the context of concentration-dependent toxicity. The risk factors for drug-induced renal injury are reasonably well understood in this population and therapeutic strategies – including judicious use of therapeutic drug monitoring – have been developed to reduce risk and facilitate safe and effective therapy. In contrast, in oncology drug-induced renal injury is also common, but the mechanism(s) and determinant(s) of risk in this setting remain much less clear than in the case of oncology. Some of

these risk factors appear to relate to age and also genetically-determined differences in drug activation and clearance. Recent research has suggested new mechanism(s) and direction(s) for better understanding the sources of variability in human drug response which determine the risk for drug-induced renal injury.

Michael Rieder

Dr. Rieder joined the faculty at Western University in London, Ontario in 1988. He obtained his MD at the University of Saskatchewan in 1980 and his Ph.D. at the University of Toronto in 1992. His paediatric resident training was at the Children's Hospital of Michigan and he completed fellowships in Paediatric Clinical Pharmacology and Paediatric Emergency Medicine at the Hospital for Sick Children and University of Toronto.

Dr. Rieder is a Professor in the Departments of Paediatrics, Physiology and Pharmacology and Medicine at Western. He is the Chair of the Section of Paediatic Clinical Pharmacology and is a Scientist with the Biotherapeutics Group at Robarts as well as a Scientist at the Children's Health Research Institute. Dr. Rieder is the Program Leader of the University of Western Ontario's Clinical Investigator Program. He is on the Scientific Program Chair of the Canadian Society for Pharmacology & Therapetucis and is the Chair of the Drug Therapy and Hazardous Substances Committee of the Canadian Society for Clinical Pharmacology. Dr. Rieder is also the representative for Western University on the Consortium for Globalization of Chinese Medicine.

Dr. Rieder has been the recipient of many awards including the 1994 and 1996 Young Investigator of the Year for the Canadian and American Societies of Clinical Pharmacology and the Senior Investigator Award of the Canadian Society of Pharmacology & Therapeutics. Other distinguished awards include the Harvard Macy Scholar Award and the Douglas Bocking Award both in 1996 and 1999 and the 2000 Teacher of the Year Award. He has won multiple teaching awards at the University of Western Ontario. He also holds the CIHR-GSK Chair in Paediatric Clinical Pharmacology, the only endowed Chair in Paediatric Clinical Pharmacology in Canada.

New Mechanisms of Drug-Induced Renal Injury

Brad Urquhart, Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, Western University

Drug-induced nephrotoxicity is an important complication of drug therapy. The spectrum of drugs that are known to cause acute kidney injury is large and spans several therapeutic areas. Drug-induced nephrotoxicity accounts for approximately 20% of cases of acute kidney dysfunction. Although there are many reports detailing acute kidney injury in adults, the incidence and prevalence in children are not well defined. Retrospective reports suggest that up to 30% of pediatric intensive care unit patients have some form of acute kidney injury. Monitoring drug-induced nephrotoxicity in children is particularly important as children with acute kidney failure are much more likely to experience chronic kidney disease later in life.

Drugs known to cause nephrotoxicity may mediate their toxic effects by multiple distinct mechanisms. Information on the identity of nephrotoxic drugs and the mechanism that they induce renal injury is an important factor in preventing drug-induced injury. In this presentation, the mechanisms by which drugs induce renal injury will be reviewed. Where appropriate, implications into the pharmacotherapy in children will be considered. Particular areas that will be discussed include changes in glomerular hemodynamics, tubular cell toxicity, rhabdomyolysis, inflammation/oxidant stress, crystal nephropathy and thrombosis. To conclude, important risk factors and methods to prevent drug-induced renal impairment will be reviewed

Brad Urquart

Dr Urquart completed his PhD in 2006 in Dr. David Freeman's laboratory. He studied homocysteine metabolism in the setting of kidney disease. It is during this time he became fascinated with effect the kidney can have on many distinct metabolic processes and how these changes can modulate drug response and toxicity. Upon completion of his PhD, Brad joined Dr. Richard Kim's lab where he studied drug transporters and the important role they play in the disposition and toxicity of drugs. In 2009, Brad was hired as Assistant Professor in the Department of Physiology and Pharmacology at Western University. Brad's independent research program investigates mechanisms that lead to altered drug

response in the setting of kidney disease. Brad has an interest in how the progression of kidney disease alters the pharmacokinetics, toxicity and therapeutic response of drugs.

When the Kidney Produces its own Poisons

Gideon Koren, The Hospital for Sick Children, Toronto, ON

Ifosfamide is a potent anticancer drug for pediatric solid tumors. While it can save lives, up to 30@ of treated children develop renal failure, characterized by both decreassed GFR and Fanconi tubulopathy. We have shown that it is a renally produced metabolite of ifosfamide, chloroacetaldehyde, that causes the renal damage, as the kidney tobular cells have the CYP enzymes to catalyze this reaction. In cell and animal model we have shown that N acetylcysteine (NAC) can mitigate this damage. PK studies have shown that systemic exposure to NAC in children is similar to the therapeutic levels effective in rats.

We have further shown that NAC does not inhibit the therapeutic anti tumor effect of ifosfamide.

This novel modality has been tried in 2 children with severe renal failure, and is now ready for controlled pediatric trials.

Gideon Koren

Gideon Koren MD, FRCPC, FACMT, is Director, The Motherisk Program, The Hospital for Sick Children, Professor of Pediatrics, Pharmacology, Pharmacy and Medical Genetics, The University of Toronto, Professor of Medicine, Pediatrics and Physiology/Pharmacology, and the Ivey Chair in Molecular Toxicology, The University of Western Ontario.

Friday 11:00 AM - Track 2

Optimizing Drug Development Using Biomarkers and Biowaivers

Approaches to Biomarkers and their Use in Drug Development: A Regulatory Perspective

Agnes V. Klein, Centre for the Evaluation of Radiopharmaceuticals and Biotherapeutic Products in the Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, ON

Biomarkers have been used in medicine to represent various stages of disease for many years. Useful examples range from older markers such as blood pressure or cholesterol levels to newer biomarkers that represent earlier stages of disease or the genetic make up of an individual that determine susceptibility to disease, response to a drug and has influence on the dosage and/or safety of a chosen therapy.
The presentation will provide a background on the approach Health Canada has taken to the science of biomarkers in the development of drugs and biologics and will provide an overview of the status and issues presented by current regulations.

Agnes V. Klein
Agnes V. Klein MD, DPH, is currently the Director, Centre for the Evaluation of Radiopharmaceuticals and Biotherapeutic Products in the Biologics and Genetic Therapies Directorate.

Dr. Klein received her medical degree from the University of Toronto. She trained in Endocrinology, Medical Biochemistry and Public and Community Health. Since joining Health Canada and the Drugs Directorate, Dr. Klein has occupied many and varied scientific and management positions within Health Canada and its regulatory arms, including having acted as the Director of the Bureau of Human Prescription Drugs. Amongst relevant accomplishments, Dr. Klein represented Health Canada on NCBHR and NCEHR and chaired the Committee on Clinical Trials of the Council.

Dr. Klein has been with the Biologics and Genetic Therapies Directorate since April 2000.

As Dr. Klein was an active participant in the CIOMS document on Pharmacogenetics and Pharmacoeconomics, this has sparked an interest in Personalized Medicine and she has championed this area for several years.

Amongst many of Dr. Klein's special interests the appropriate design of clinical trials and the various and complex ethical issues attendant to the design and conduct of clinical trials and other studies in human subjects stands out. Dr. Klein is a member of Health Canada's Research Ethics Board.

ATP Metabolism as Biomarker Target for Drug Development

Pollen K.F. Yeung, Professor of Pharmacy and Medicine, Dalhousie University, Halifax, NS, Canada

Although clinical drug development has made significant stride along with pharmaceutical sciences over the last 3 decades, from the application of pharmacokinetics in the 1970's, controlled clinical studies for efficacy in 1980's, pharmacodynamics and pharmacogenetics in the 1990s, to a focus on drug safety in the current decade, the success rate to introduce new effective and safe therapeutic agents has not kept up with expectations from the financial investment and those of patients. In another word, there is inadequate improvement in drug therapy nor financial reward. Identification and application of biomarkers for lead selection and optimization has been heralded as one of the most likely scientific approach to increase the success of drug development. It is widely conceived that biomarkers are the scientific basis and effective tools guiding drug development and regulatory decisions. The

era of application of biomarkers in drug development began in the early turn of this century and is now becoming a main stream in pre-clinical and clinical drug development especially with the increasing use of diagnostic tests. According to the Pharm 2010 document from the IBM Consulting Service, biomarkers can create a business model which could be used to revitalize the much-needed impetus in novel drug development in the pharmaceutical and biotechnology industry. The presentation will focus on the potential of using ATP metabolism as biomarker target for cardiovascular protection and toxicities. It will also discuss the opportunities, challenges and obstacles of exploiting ATP metabolism as targets for drug development and personalized medicine, and how government and regulatory and funding agencies may expedite to advance the course of biomarker discovery and development in Canada and globally.

Pollen Yeung
Dr. Yeung is currently Professor of Pharmacy and Medicine in Dalhousie University (Halifax, NS, Canada). His research interest is in the area of pharmacokinetics, metabolism and biomarker assessment or cardiovascular and anti-cancer drugs. He is an active contributor and consultant for many academic and professional organizations, pharmaceutical industry, granting councils and the Government. He is currently a member on the Board of the Canadian Society of Pharmaceutical Sciences (CSPS), a member on the Editorial Board for Recent Review of Clinical Trials, Drug Metabolism Open Journal, and Metabolites. He was the Vice Chair of the Cardiovascular, Pulmonary and Renal (CPR) Section of American Society of Clinical Pharmacology and Therapeutics (ASCPT) from 2006 -2010, a member of the Board of Biotechnology Life Sciences Industry Association in Nova Scotia (BioNova) from 1999 – 2007, Scientific Officer for Nova Scotia Health Research Foundation from 2009 - 2011, Member of the Scientific Advisory Committee on Oncology Therapies for Health Canada from 2004 – 2007.

The Role of Biowaivers in Drug Development

Raimar Löbenberg, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Computer simulations gained more and more importance in modern drug development to aid in the establishment of in vitro in vivo correlations (IVIVC). IVIVCs are important for product lifecycle management and to set product specifications. The early knowledge of product performance is key for a successful clinical development. The talk will show how physiologically based simulation models can be used to predict the bioavailability of drugs. Examples will be given how these models can be used to predict disease state performance of dosage forms.

Raimar Löbenberg
See Bio page 78.

Modified Release Formulations: Waiver for Bio-studies for Different Dosage Strengths of a Drug Product Manufactured for Global Market

Jasmina Novakovic and Yu Chung Tsang, Apotex Inc, Toronto, Ontario, Canada

Demonstration of bioequivalence (BE) is commonly required for regulatory approval of a generic (test) modified release product and the testing has to be performed against the market-specific reference listed drug (RLD). Depending on the jurisdiction, additional BE studies may be needed for multiple strengths of a MR product even if they are similarly formulated. These BE studies can significantly raise the development cost of a generic product destined for global market. Development of In Vitro/ In Vivo correlations (IVIVC) to predict bioavailability (BA) and/or BE of drug products based on their in vitro dissolution data is encouraged by the regulatory authorities but its utilization has been scarce. The objective of this presentation is to illustrate the applicability of IVIVC approach to: (a) predict BA/BE of MR products and the RLDs for different market-places; (b) support waiver of BE studies for different strengths of a MR product. Controlled-release formulations of various strengths of an RLD with dose-independent pharmacokinetics manufactured for three different marketplaces employing the same release mechanism and the

same excipients are used for illustration. A Level A IVIVC model was developed and validated by employing in vitro dissolution profiles and in vivo PK data for the test formulations of different release rates as per the FDA IVIVC guidance. The predictive power of the established IVIVC was assessed for internal and external predictability. The PK profile of different strengths of the RLDs from different marketplaces were generated in some BE studies of the test product, as well as predicted by the established IVIVC. The differences between the observed and predicted means of AUC and Cmax were ≤10%. Therefore, the IVIVC model provided adequate prediction of BA for the RLDs. Finally, comparisons of deconvoluted PK profiles revealed similarities of the RLDs from different marketplaces and supported BE of various strengths of the test product and the corresponding RLDs. In conclusion, a validated Level A IVIVC was shown to be an useful tool to predict BA/BE of MR test products and the RLDs for different market-places. It can significantly reduce the number of BE studies required and thus, the cost of drug development for global market approval.

Jasmina Novakovic

Dr Jasmina Novakovic has been employed as a Scientific Leader at Apotex Inc since 2007. Jasmina is covering multidisciplinary areas of pharmaceutical research and development, with special focus on bio-pharmaceutical modelling to support product development and biowaivers. A pharmacist by training, she holds PhD in Analytical Chemistry (Charles University, Prague) and in Pharmaceutical Chemistry (University of Belgrade, Serbia). Her post-doctoral fellowship in the area of pre-clinical testing of novel drug molecules, Jasmina conducted under supervision of Drs Thiessen and Spino at the University in Toronto, Faculty of Pharmacy (2000-2004). She was employed as a Research Associate at the same laboratory (2004-2007) prior to joining Apotex Inc in 2007. In addition to her industrial employment, Jasmina is teaching pharmaceutical subjects at the Faculty of Continuing Education, Seneca College at York University.

Friday 11:00 AM - Track 3

CSPS Trainee Oral Presentations

[Presentations TBA]

Friday 11:00 AM - Track 3

CPS Oral Presentations

Hydrogen Sulfide *S*-sulfhydrates Pyruvate Carboxylase and Stimulates Gluconeogenesis

Yang, Guangdong, Lakehead University

Background/objectives: Cystathionine gamma-lyase (CSE)-derived H_2S is a candidate for regulation of glucose metabolism and insulin secretion. H_2S *S*-sulfhydration is now proposed as a mechanism for H_2S-mediated signaling. Pyruvate carboxylase (PC) is an important enzyme for gluconeogenesis. *S*-sulfhydration regulation of PC by H_2S and its implication in gluconeogenesis in liver have been unknown.
Results: In the present study, we demonstrated that H_2S stimulates PC activity in a time and dose-dependent manner in HepG2 cells (a human hepatocellular liver carcinoma cell line). CSE overexpression increased H_2S production and induced PC activity. We further found that both CSE overexpression and exogenously applied H_2S strengthen gluconeogenesis. H_2S had little effect on the expressions of PC mRNA and protein, while H_2S *S*-sulfhydrated PC in a dithiothreitol-sensitive way. CSE overexpression also significantly induced PC *S*-sulfhydration.
Methods: CSE expression was measured by Real-time PCR and western blotting. PC *S*-sulfhydration was measured by a modified biotin assay. Gluconeogenesis and H_2S production were determined by enzymatic assay, respectively.
Conclusions: H_2S stimulates gluconeogenesis in liver cells possibly through *S*-sulfhydration regulation of PC. The finding will help reveal a novel physiologic posttranslational modification for proteins by *S*-sulfhydration, which potentially influence a multitude of biological pathways.

Cardiac Specific Over-Expression Of Membrane-Associated Human Stem Cell Factor Promotes Epicardial Activation Post Myocardial Infarction

Fu-Li Xiang[1], Xiangru Lu[3]*, Yin Liu[1]*, Murong Liu[3]*, Qingping Feng[1,2,3], Departments of [1]Physiology and Pharmacology, and [2]Medicine, University of Western Ontario, [3]Lawson Health Research Institute, London, Ontario, Canada.

Background: Myocardial infarction (MI) is one of the leading causes of death worldwide. Our previous study demonstrated that the cardiac-specific over-expression of human membrane-associated stem cell factor (M-hSCF) improves cardiac function and survival by increasing circulating stem cell retention. The aim of the present study was to investigate the effects of cardiomyocyte-specific overexpression of M-hSCF on epicardial activation post-MI.
Methods and Results: Wild-type (WT) and the inducible cardiac-specific M-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. Artery density was significantly increased in the peri-infarct area of hSCF/tTA mice compared to WT 5 days post-MI ($P<0.05$). Wt1 lineage tracing using $ROSA^{mTmG}$;$Wt1^{CreER}$ mice showed enhanced $Wt1^{+}$ cell-derived EPDCs migration into infarcted myocardium in Ad-hSCF intra-myocardium injection group compared to Ad-LacZ 5 days post-MI ($P<0.05$). In vitro, Proliferation was significantly enhanced in Ad-hSCF infected EPDCs compared to Ad-GFP 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 infected with Ad-hSCF induced more EGFP+ EPDCs seeded in the upper compartment to migrate through the trans-well membrane compared to Ad-LacZ after twenty-four hour culture ($P<0.05$). The effects of M-SCF expression on EPDC proliferation and migration

were abrogated by blocking the *ckit* signaling using ACK2 treatment.
Conclusions: Cardiomyocyte-specific over-expression of M-hSCF promotes the activation and migration of EPDCs post-MI.

Atrial Fibrillation is Associated with Increased K_{ATP} Channel Function in RGS4-deficient Mice

Carlo Cifelli[1], Alexandra S. Mighiu[1], Hangjun Zhang[1], and Scott P. Heximer[1,2]. [1]Department of Physiology, University of Toronto; [2]Heart and Stroke/Richard Lewar Centre of Excellence, University of Toronto

Background/objectives: Atrial fibrillation (AF) is a major cause of morbidity and mortality. Atrial ATP-sensitive K^+ (K_{ATP}) channels can provoke the development of ventricular arrhythmias, however very little is known about the role of these channels in initiating and perpetuating atrial arrhythmias. Since K_{ATP} channels are direct effectors of the heterotrimeric $G\alpha_{i/o}$ proteins and RGS4 is a potent inhibitor of $G\alpha_{i/o}$ signaling, we here investigate whether altered expression of RGS4 modifies K_{ATP} channel function in a manner that enhances susceptibility to AF.
Methods and Results: Susceptibility to AF was characterized using optical mapping of intact atrial tissues using a voltage sensitive dye. RGS4-deficient mice readily develop re-entrant rotors in the presence of the muscarinic receptor agonist carbachol and rapid atrial pacing, while the same intervention could not induce AF in control wild-type mice. Furthermore, K_{ATP} channel blockade with glibenclamide reversed the sensitivity of RGS4-deficient atria to AF suggesting that increased K_{ATP} channel activity may contribute to atrial remodeling in our experimental model of AF. Consistent with these findings, quantitative real-time PCR revealed that elderly (>75 weeks) RGS4-knockout mice have increased mRNA expression of Kir6.2 (> 10-fold) and SUR1 compared to young (16 week) RGS4-knockout and wild-type mice. In parallel, Kir6.2 subunit expression is increased in dogs following 6 weeks of atrial tachypacing (a model of chronic AF).
Conclusions: Taken together, these results suggest that enhanced sensitivity of K_{ATP} channels in RGS4-deficient atria may contribute to the progression of AF in these animals. Future studies will involve the use of Kir6.2-knockout and RGS4/Kir6.2 double knockout mice to further characterize the relative contribution of K_{ATP} channels to electrophysiological remodeling in our model of AF.

Localized Elevation of Cytosolic Free Calcium is Required for Uropod Retraction and Osteoclast Migration

Wheal BD, Tanabe N, Dixon SJ, Sims SM. [*]Non-member of CPS; [1]Graduate Program in Neuroscience, The University of Western Ontario, London, Canada; [2]Nihon University School of Dentistry, Tokyo, Japan; [3]Department of Physiology and Pharmacology, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, Canada

Background/Objectives: Osteoclasts are large multinucleated cells responsible for the resorption of bone and other mineralized tissues. Mature osteoclasts are highly motile and alternate between cycles of bone resorption and migration. However, little is known regarding the subcellular mechanisms that regulate osteoclast motility. We hypothesized that changes in the concentration of cytosolic free calcium ($[Ca^{2+}]_i$) contribute to the control of osteoclast migration. Our purpose was to characterize subcellular changes in osteoclast $[Ca^{2+}]_i$ and their possible role in regulating osteoclast motility.
Methods: $[Ca^{2+}]_i$ was monitored by digital fluorescence imaging of fura-2-loaded osteoclasts using alternating excitation wavelengths of 345/380 nm with emission at 510 nm.
Results: Migrating osteoclasts exhibited a polarized morphology with lamellipodia extending forward at the leading edge of the cell and the uropod undergoing retraction at the rear, generating net forward movement. Migrating osteoclasts displayed a distinct spatiotemporal pattern of $[Ca^{2+}]_i$ that consisted of localized elevation of $[Ca^{2+}]_i$ in the trailing edge of the cell that coincided with uropod retraction. This elevation of $[Ca^{2+}]_i$ was blocked in osteoclasts loaded with the cytosolic calcium chelator BAPTA. Time-lapse recordings revealed that BAPTA-loaded osteoclasts continued to extend lamellipodia but failed to detach from the substrate, giving rise to dramatically elongated, highly branched morphologies.
Conclusion: These findings reveal a novel spatiotemporal pattern of $[Ca^{2+}]_i$ in osteoclasts, with a localized elevation of $[Ca^{2+}]_i$ being required for uropod retraction. This study reports a heretofore

unrecognized role for subcellular Ca^{2+} signaling in the regulation of osteoclast migration.
Supported by the Canadian Institutes of Health Research (CIHR).

Genome-Wide Rnai Screening Identifies Drosophila Bestrophin 1 As A Swell-Activated Chloride Channel

Stotz, Stephanie C.*, Keating, Myles B.*, and Clapham, David E.*, Department of Cardiology, Children's Hospital, Boston; Department of Neurobiology, Harvard Medical School; Howard Hughes Medical Institute

Background: Chloride channels (Cl_{swell}) activated by cell swelling or volume increases are ubiquitous. Their activity contributes to the maintenance of intracellular osmolality and prevents catastrophic cell rupture. Despite intensive efforts to identify the protein responsible for the mammalian Cl_{swell} channel, its identity remains unknown.
Methods: We have completed an unbiased genome-wide RNAi screen using *Drosophila* S2R+ cells to identify new Cl_{swell} candidates and regulators. A genetically engineered anion-sensitive YFP reported anion influx through active Cl_{swell} channels with fluorescence suppression. RNAi targeting the Cl_{swell} channel or essential regulating molecules prevented substantial fluorescence suppression.
Results: Our RNAi screen identified *Drosophila* Bestrophin 1 as a leading candidate for a Cl_{swell}. RNAi specific to *dBest1* eliminated the D*rosophila* $I_{Clswell}$, as did over-expression of a dominant negative *dBest1* mutant channel. In contrast, exogenous expression of *dBest1* in HEK cells resulted in a $I_{Clswell}$ that closely resembled the *Drosophila* $I_{Clswell}$. Mouse Best2 (mBest2), the closest mammalian ortholog of dBest1, is swell insensitive until its N-terminus is switched with that of dBest1. The chimera maintains mBest2-like pore properties, evidence that the protein itself is a channel rather than an essential auxiliary regulator.
Conclusions: Genome-wide RNAi screening using an anion sensitive fluorescent reporter has identified dBest1 as the *Drosophila* Cl_{swell} channel. dBest1, in the absence of other *Drosophila* proteins, forms a channel clearly responsive to swell, confirming findings of the Hartzell lab. Its activation is dependent upon the character of its N-terminus. Swell sensitivity can be conferred to the mammalian homolog with the switch of a single domain.

Poster Presentations
Day 1
Wednesday, June 13, 2012

CSPS Posters - Day 1

Wednesday, June 13, 2012

Biomedical Sciences

1. In situ Generated (E)-2,4-diene-valproic Acid Contributes to the Toxicity of (E)-2-ene-valproic Acid in Sandwich-cultured Rat Hepatocytes Pretreated with Phenobarbital

Jayakumar Surendradoss, Thomas K.H. Chang, and Frank S. Abbott. Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC, Canada.

Purpose: Formation of reactive metabolites like (*E*)-2,4-diene-VPA is a proposed mechanism for the idiosyncratic hepatotoxicity of valproic acid (VPA). (*E*)-2,4-diene-VPA is formed by cytochrome P450 (CYP)-mediated desaturation of (*E*)-2-ene-VPA or mitochondrial β-oxidation of 4-ene-VPA, which itself is a CYP-catalyzed metabolite of VPA. (*E*)-2,4-diene-VPA is reactive and more hepatotoxic than VPA, but direct experimental evidence is needed to evaluate the effect of the *in situ* generated metabolite on VPA hepatotoxicity.
Methods: We assessed the effect of modulating the *in situ* formation of (*E*)-2,4-diene-VPA by pretreatment with phenobarbital (PB, a CYP inducer) and 1-aminobenzotriazole (1-ABT, a CYP inhibitor) on markers of oxidative stress, steatosis and necrosis in sandwich-cultured rat hepatocytes treated with VPA or (*E*)-2-ene-VPA.
Results: PB increased the metabolism of (*E*)-2-ene-VPA to (*E*)-2,4-diene-VPA, and this was accompanied by enhanced toxicity of (*E*)-2-ene-VPA, whereas 1-ABT attenuated the increase in the levels of the (*E*)-2,4-diene-VPA metabolite and toxicity by PB. Neither PB nor 1-ABT affected (*E*)-2,4-diene-VPA formation and toxicity in VPA-treated hepatocytes.
Conclusion: *In situ* formed (*E*)-2,4-diene-VPA contributes to the toxicity in sandwich-cultured rat hepatocytes, if generated at sufficiently high levels.
Acknowledgements: This research was supported by the Canadian Institutes of Health Research (Grant MOP-13744) and Michael Smith Foundation of Health Research (senior scholar award to T.K.H.C.). The essential data in this poster was previously presented at the Experimental Biology 2012 meeting in San Diego, April 21-25, 2012.

2. Novel Dehydroepiandrosterone Derivatives as Antiandrogens

Marisa Cabeza[a], Yazmín Arellano, Araceli Sánchez, Yvonne Heuze[a], and. Eugene Bratoeff[b]. Department of Biological Systems and Animal Production Metropolitan University-Xochimilco[a], Mexico D. F., Mexico; Department of Pharmacy, Faculty of Chemistry[b], National University of Mexico City, Mexico D. F., Mexico.

Purpose: The aim of these studies was to synthesize different ester derivatives of dehydroepiandrosterone with therapeutic potential as antiandrogens.
Methods: The biological effect of these steroids was demonstrated as *in vivo* as *in vitro* experiments. In the *in vivo* experiments, we measured the activity of 12 steroids on the diameter of the pigmented spot of the flank organs as well as the weight of the prostate of gonadectomized hamsters treated with testosterone. For the studies *in vitro*, we determined the IC_{50} values by measuring the concentration of the steroidal derivatives that inhibits 50% of the activity of 5α-reductase type 1 and 2 which are present in rat liver and human prostate respectively. The binding capacity of these derivatives to the androgen receptors (AR) obtained from rat's prostate cytosol was determined also.
Results: The results from these experiments indicated that all compounds significantly decreased the diameter of the pigmented spot of the flank organs. However only compounds 5-androsten-3β-benzoato-17-ona **1**, 5-androsten-3β-(4-clorobenzoato)-17-ona **3** and 5-androsten-3β-(4-yodobenzoato)-17-ona **5** reduced the weight of prostate as compared to testosterone treated animals and this reduction of the weight of this gland was comparable to that produced by finasteride. On the

other hand, all novel steroids inhibited the activity of human 5α-reductase enzyme type 1; but only steroids **1** and **3** inhibited type 2, 5α-reductase. However, none of these compounds binds to the AR.
Conclusion: The compounds containing an ester moiety in C-3 in the androstane skeleton, showed pharmacological activity, since reduced the diameter of the pigmented spot of hamster' flank organs. Furthermore, all the studied compounds inhibited the activity of 5α-reductase type 1, but did not bind to the AR.

3. Molecular Interactions of Natural and Synthetic Steroids in Female Hamsters' Flank Organs

Marisa Cabeza[a], Barak Naranjo[a], Yvonne Heuze[a], Araceli Sánchez[a], Mercedes Hernández[a]; Teresita Sainz[a], Eugene Bratoeff[b]. Department of Biological Systems and Animal Production Metropolitan University-Xochimilco[a] Mexico D.F., Mexico; Department of Pharmacy, Faculty of Chemistry[b], National University of Mexico City, Mexico D.F., Mexico

Background: The initial step of steroidal action on target cells is gene activation; therefore, the quantification of mRNA is a direct method for comparing the role of different steroids in the skin.
Purpose: This study demonstrated the role of several steroids on the mRNA expression encoding for different enzymes involved in the lipid metabolism in hamsters' flank organs, which are a pilosebaceous complex.
Methods: To determine the effect of treatments with testosterone (T) progesterone (P), levonorgestrel (LNG), 17α-p-chlorobenzoyloxy-6-chloropregn-4,6-diene-3,20-dione (**5)** and 17α-p-chlorobenzoyloxy-4,6-pregnadiene-3,20-dione (**6);** T and/or LNG; T and **5** or **6**; P and/or **5** or **6** on the expression of mRNA encoding for lipid enzymes, the steroids were applied to the glands; later, the mRNAs expression for the enzymes was determined by PCR. The binding of **5** and **6** to the progesterone receptor (PR) was also evaluated.
Results: Treatments with T, LNG, T+LNG, P, T+P, **5**, T+**5,** T+**6,** P, P+**5** and P +**6** increased the mRNA expression for glycerol 3-phosphate acyl transferase (GPAT), β-hydroxy-β-methylglutaryl-CoA synthase (HMG-CoA-S), β-hydroxy-β-methylglutaryl-CoA reductase (HMG-CoA-R), phosphatidilinositol synthase as compared to the controls. However, squalene synthase was increased with all treatments except with T+**5** and **6**; **6** did not significantly increase the expression for GPAT or HMG-CoA-S, however it increased the concentration of HMG-CoA-R enzyme. **5** and **6** bind to the PR, thus indicating that the effect of these steroids on the mRNA expression could be the result of their binding.
Conclusion: The lipid metabolism is regulated by several steroids thought different mechanism of action, in flank organs.

4. Role of ATP-binding Cassette (ABC) Transporters in the Permeability of Antiretroviral Drugs (ARVs) at the Blood-Testis Barrier

Kevin Robillard[1], Md. Tozammel Hoque[1], Guijin Zhang[2], Charles la Porte[2 3] and Reina Bendayan[1]. [1]Graduate Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON; [2]Ottawa Hospital Research Institute, Ottawa Hospital, Ottawa, ON; [3]University of Ottawa, Ottawa, ON, Canada

Purpose: The blood-testis barrier (BTB), primarily composed of Sertoli cells, is responsible for protecting developing germ cells from xenobiotic exposure. ABC membrane-associated drug efflux transporters, P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and the multidrug resistance-associated proteins (MRPS), have been shown to restrict ARVs permeability at blood-tissue barriers such as the blood-brain barrier. However, it remains unclear if these transporters are functional at the level of Sertoli cells and can regulate ARVs permeability at the BTB. We investigated the functional expression of ABC transporters in a mouse Sertoli (TM4) cell system, in primary cultures of Human Sertoli cells (HSEC) and in wild-type and triple knock-out (abcb1a/1b-/-, abcg2-/-) (TKO) mice.
Methods: Quantitative real-time PCR (qPCR), immunoblotting analysis and immunofluorescence microscopy were applied to determine drug transporter mRNA, protein expression and cellular localization, respectively, in TM4 and HSEC monolayers. Functional assays using specific radiolabelled and fluorescent substrates for each of the transporters of interest: P-gp (rhodamine-6G (R6G) and atazanavir, a HIV protease inhibitor), Bcrp (mitoxantrone), Mrps (2',7'-bis-(2-

carboxyethyl)-5-(and-6)–carboxyfluorescein, BCECF) were performed to characterize transporter activity and ARVs accumulation by TM4 cell monolayers. *In vivo* tissue distribution studies using [^{3}H]-atazanavir, a HIV protease inhibitor, were undertaken in wild type and TKO mice following tail-vein injection.

Results: Expression of Mdr1b/MDR1/P-gp, Mrp1/MRP1, and Mrp4/MRP4 was confirmed by qPCR and immunoblotting analysis in TM4 and HSEC. Immunofluorescence studies revealed plasma localization of P-gp, Mrp1/MRP1 and Mrp4/MRP4 in both cell systems. Accumulation of i) R6G, ii) [^{3}H]-atazanavir, iii) BCECF, and iv) [^{3}H]-mitoxantrone by TM4 cell monolayers in the presence of established inhibitors demonstrated that these transporters are functional. In addition, several ARVs significantly enhanced the accumulation of R6G, [^{3}H]-atazanavir, BCECF and [^{3}H]-mitoxantrone by TM4 cells. Data from *in vivo* tissue distribution studies demonstrated a significant ($p<0.05$) increase in testicular tissue/plasma ratio of atazanavir in TKO mice (0.37 ± 0.04) compared to control wild-type mice (0.20 ± 0.01).

Conclusion: *In vitro* data suggest that ABC transporters are functional in both human and rodent Sertoli cells. Findings from the *in vivo* tissue distribution studies suggest that these transporters are involved in limiting the permeability of the HIV protease inhibitor atazanavir within the testes. Taken together, these data imply that efflux drug transporters play an important role in the distribution of ARVs in the male genital tract, a site for HIV infection and transmission.

Supported by the Ontario HIV Treatment Network (OHTN).

5. Effect of Anti-inflammatory Compounds on HIV-1 gp120 -mediated Brain Inflammation

Tamima Ashraf, Wenlei Jiang and Reina Bendayan. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada

Purpose: Cognitive impairment remains highly prevalent in HIV-1 infected patients due to viral replication and associated inflammation in the brain. One obstacle to effective treatment is poor brain penetration of antiretroviral drugs due to functional expression of efflux transporters [P-glycoprotein (P-gp) and Multidrug-resistance associated proteins (MRPs)]. Identifying therapeutic compounds that are not substrates of these transporters but target signaling pathways involved in inflammation may benefit treatment of HIV-associated neurological complications. Our laboratory has previously established that signaling pathways, NF-kB and JNK, are involved in HIV-1 gp120 associated inflammatory response and regulation of efflux transporters in *in vitro* glial cell systems. In this study, by intracerebroventricular administration of HIV-1 gp120 in rats, we implemented an *in vivo* model of brain inflammation and investigated the regulation of transporters as well as the anti-inflammatory properties of minocycline, chloroquine and simvastatin. Using this model, we also intend to elucidate key signaling pathways involved in HIV-associated brain inflammation *in vivo*.

Methods: Male Wistar rats were administered a single dose of $gp120_{ADA}$ (500ng) daily for 7 consecutive days intracerebroventricularly with or without prior intraperitoneal administration of minocycline or chloroquine or simvastatin. Real-time qPCR was used to determine gene expressions of inflammatory markers in different brain regions (frontal cortex, hippocampus, striatum). Cytokine secretion in cerebrospinal fluid (CSF) was measured using ELISA. Protein expression of P-gp and Mrp1 was detected using immunoblot analysis.

Results: In $gp120_{ADA}$-injected rats, transcripts of TNF-α, IL-1β and iNOS were significantly elevated in frontal cortex and hippocampus, whereas, elevated IL-1β and iNOS mRNA were observed in striatum. In CSF, a significant increase in TNF-α and IL-1β was detected. In addition, consistent with our previous *in vitro* findings, gp120 administration resulted in a decrease in P-gp expression and an increase in Mrp1 in several brain regions. Furthermore, minocycline or chloroquine completely attenuated the upregulation of IL-1β and iNOS transcripts in all three brain regions and prevented IL-1β secretion in CSF. Minocycline was also successful in suppressing TNF-α transcripts in brain tissues and prevented TNF-α secretion. Simvastatin attenuated both IL-1β and iNOS transcripts in hippocampus and striatum, but only suppressed iNOS in the frontal cortex.

Conclusion: Our data demonstrate that administration of HIV-1 $gp120_{ADA}$ in rodents generates an inflammatory response and alters expression of efflux transporters in several regions of the brain. Furthermore, minocycline and chloroquine can reverse gp120-associated brain inflammatory responses suggesting that these agents

could potentially be considered in the prevention/treatment of HIV-associated cognitive disorders.

Supported by operating grants from OHTN and CIHR. Dr. Bendayan is a Career Scientist from the OHTN.

6. Organic Anion Transporting Polypeptides (OATPs): A New Molecular Target for Hormone Dependent Breast Cancers

Nilasha Banerjee, Humphrey Fonge, Christine Allen and Reina Bendayan. Leslie Dan Faculty of Pharmacy, University of Toronto

Purpose: The purpose of this study is to explore the potential of Organic Anion Transporting Polypeptides (OATPs), a family of membrane associated uptake transporters, as a novel molecular target for breast cancers. Estrone-3-sulphate (E3S), an OATP substrate, is the predominant source of tumor estrogen in post menopausal hormone dependent breast cancer patients. Several OATP isoforms are over expressed (up to 10 times) in breast cancer tissues, suggesting their potential contribution towards the 2-3 times higher tumoral concentration of E3S. By exploring the differential expression and function of OATPs in breast epithelial and breast cancer cells and by investigating the pharmacokinetic and bio-distribution profile of E3S, in tumour (hormone dependent and independent) bearing mice models, the potential for developing OATPs as a novel molecular cancer target and E3S as a targeting ligand for hormone dependent post-menopausal breast cancer patients can be established.
Methods: Gene and protein expression of seven OATPs that recognize E3S as a substrate, were compared in normal breast epithelial cells (MCF10A), hormone dependent (MCF7) and hormone independent breast cancer cells (MDA/LCC6-435, MDA-MB-231, MDA-MB-468) by qPCR and immunoblotting. Time course based transport studies for determining specificity of OATP mediated E3S uptake were performed in the presence or absence of transport inhibitor, 100μM Bromosulphophthalein (BSP). Kinetics of E3S transport were determined by Michaelis–Menten kinetics through concentration dependent uptake studies. Pharmacokinetics and distribution of E3S was determined *in-vivo*, by measuring ^{3}H-E3S concentration in plasma, tissues and tumour xenografts (MCF7 and MDA-MB-231) implanted in mice models.
Results: Gene expression of SLCO1A2, 1B1, 1B3, 2B1 and 3A1 was exclusive, similar or significantly higher in cancer cells compared to MCF10A. Protein expression of OATPs was either exclusive or higher in cancer cells compared to MCF10A. Furthermore, specificity of OATP mediated E3S uptake was observed only in cancer cells, with 10 times greater transport efficiency in MCF7 cells, than in the hormone independent cells. *In-vivo* studies revealed a significant tumour uptake 6 h post injection, reaching 10 and 14 %ID/g (%Injected Dose/g) tumour in the MDA-MB-231 and MCF-7 tumour-bearing mice, respectively. High tumour to blood ratio (9 for MDA-MB-231; 7 for MCF7 xenografts), was observed 48 h post injection.
Conclusion: Taken together, these data suggest that OATPs could be a potential molecular target, and E3S could serve as a novel ligand for active targeting of hormone dependent breast cancers in post-menopausal patients.

7. Molecular Interactions of GR and Liver X Receptor β (LXRβ) Play a Critical Role in Glucocorticoid Induced Gluconeogenesis and Hepatosteatosis

Rucha Patel[1], Ricky Tsai[1], Arturo Orellana[2], Carolyn L.Cummins[1]. [1]Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario; '[2]Department of Chemistry, York University, Toronto, Ontario, Canada.

Introduction: Serum glucocorticoids (GCs) are elevated in the patients with type II diabetes and in diabetic mouse models. Synthetic GCs (e.g. dexamethasone (DEX)) are potent anti-inflammatory drugs but their long term use cause deleterious side effects such as hyperglycemia, fatty liver, insulin resistance and type II diabetes. GR and LXRs are members of the nuclear hormone receptor family that regulate overlapping target genes involved in gluconeogenesis, lipogenesis and inflammation. Recently, we have reported that the whole body LXRβ−/− mice are protected against GC induced hyperglycemia and hepatosteatosis without affecting GC mediated suppression of inflammation [Patel, R et al. 2011]. We hypothesized that resistance to GCs was primarily due liver specific loss of LXRβ (and not other peripheral tissues). Furthermore, we hypothesize that LXRβ antagonists will be able to

dissociate the anti-inflammatory effects of GCs from the metabolic effects.

Methods and Results: Herein, we show that 5 day twice daily DEX (5mg/kg) treatment to $LXR\beta^{fl/fl}$ control and liver specific LXR-null mice ($Alb^{+/cre}LXR\beta^{fl/fl}$: liv.LXRβ) caused hyperglycemia and hepatosteatosis only in control mice but not in the liv.LXRβ−/−. In agreement, time course studies in primary hepatocytes demonstrated that DEX treatment caused potent induction of Pepck mRNA in the WT and LXRα−/− but not in the LXRβ−/− and LXRαβ−/−. Co-IP studies showed a physical interaction between LXRβ and GR in liver cells. Interestingly, antagonizing LXRβ activity (with cmp17) along with DEX co-treatment in perfused LXRα−/− livers and primary hepatocytes reduced DEX-mediated Pepck induction, stabilized LXRβ recruitment to the Pepck promoter and changed the chromatin conformation from an open to closed conformation (as measured by decreased deacetylation of histone 4 by chromatin immunoprecipitation).

Conclusion: The in-vivo studies in the liv.LXRβ−/− confirmed that protection against GC-induced hyperglycemia and hepatosteatosis is due to liver LXRβ and not related to alteration of hormonal regulation in other metabolic organs. The LXR antagonist and DEX co-treatment studies demonstrated that deleterious side-effects of GC may be avoided by selectively modulating the nuclear receptors LXRβ and GR through control of the key gluconeogenic gene Pepck.

8. Inhibition of Soluble Epoxide Hydrolase Confers Cardioprotection against Isoproterenol-Induced Cardiac Hypertrophy

Hassan N. Althurwi[1], Beshay N.M. Zordoky[1], Bruce D. Hammock[2] and Ayman O.S. El-Kadi[1]. [1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada; [2]Department of Entomology and Cancer Research Center, University of California, Davis, CA.

Purpose: We have previously shown that isoproterenol-induced cardiac hypertrophy causes significant changes to several cytochromes P450 (CYP) and soluble epoxide hydrolase (sEH) gene expression. These changes have led to higher production of the cardiotoxic 20-hydroxyeicosatetraenoic acid (20-HETE) metabolite and lower production of the cardioprotective epoxyeicosatrienoic acids (EETs) metabolites in the hypertrophied hearts. Therefore, it is important to examine whether the inhibition of sEH by 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea (TUPS) will protect against isoproterenol-induced cardiac hypertrophy.

Methods: Male Sprague–Dawley rats were treated daily for seven days with either TUPS (oral gavage, 0.65 mg/kg/day), isoproterenol (intraperitoneal, 5 mg/kg/day), or the combination of both. TUPS administration was started 24 hours prior to isoproterenol administration and continued concurrently afterward. Thereafter, the heart was harvested and the heart to body weight ratio was measured, and the expression of hypertrophic markers, fibrotic markers and different *CYP* genes were determined by real time-polymerase chain reaction (qRT-PCR).

Results: Our results showed that isoproterenol alone caused a significant induction of the hypertrophic markers, fibrotic markers as well as the heart to body weight ratio. In addition, isoproterenol treatment caused an induction of *CYP1A1*, *CYP1B1, CYP2B1, CYP2B2, CYP4A3* and *CYP4F4* gene expression, and an inhibition of CYP2E1. On the other hand, treatment with TUPS significantly reduced the isoproterenol-mediated increase of heart to body weight ratio by 35%. In addition, TUPS significantly inhibited the isoproterenol-mediated induction of hypertrophic markers, ANP, β-MHC and BNP by 37%, 50% and 23%, respectively, as well as fibrotic markers, procollagen(I), procollagen(III) and TGF-1 by 32%, 25%, and 46%, respectively. Furthermore, TUPS significantly reduced the isoproterenol-mediated induction of *CYP1B1, CYP1A1, CYP2B1, CYP2B2, CYP4A3* and *CYP4F4* gene expression. In a different manner, there was no significant change in *CYP2E1* expression by TUPS.

Conclusion: TUPS partially protects against isoproterenol-induced cardiac hypertrophy which further confirms the role of sEH and CYP enzymes in the development of cardiac hypertrophy.

Acknowledgements: This work was supported by a grant from the CIHR to A.O.S.E. H.N.A is the recipient of Saudi Government Scholarship.

9. The Cardioprotective Effect of 11,12 EET Against Isoproterenol-induced Cardiac Hypertrophy in the Rat Cardiomyoblast, H9c2 Cells

Mandy M.Y. Tse, Ghada Abdelhamid, and Ayman O.S. El-Kadi. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Purpose: Cardiac hypertrophy is one of the well established risk factors for heart failure and sudden death. It can be defined as an increase in heart mass in response to stress stimulus. The role of cytochrome P450s (CYP) has been extensively investigated in understanding the development of cardiac hypertrophy. CYP epoxygenases (CYP2B1/2C11/2J3) metabolize arachidonic acid into the cardioprotective metabolites, 5,6- 8,9-, 11,12- and 14,15-epoxyeicosatrienoic acids (EETs). The EETs can be further metabolized by soluble epoxide hydrolase into a less biologically active form, dihydroxyeicosatrienoic acids (DHETs). On the other hand, CYP ω-hydroxylases (CYP1A1/1B1/4F1) metabolize arachidonic acid into cardiotoxic metabolites, 17-, 18-, 19-, 20-hydroxyeicosatetraenoic acids (HETEs). In this study, we examined the protective effect of 11, 12-EET on isoproterenol-induced cardiac hypertrophy and its modulation of cytochrome P450 enzymes in H9c2 cell line.
Methods: Isoproterenol at different concentrations (1, 10, 50 and 100 μM) were added to the medium of H9c2 cells for 24 and 48 hrs. Total RNA was isolated using Trizol. Reverse transcription was performed and quantitative real-time PCR was used to determined mRNA expression. To determine the protective effects of 11,12-EET, H9c2 cell were incubated with isoproterenol at a concentration of 100 μM in the absence and presence of 11,12-EET at a concentration of 1 μM for every 8hrs for 24hrs.
Results: Isoproterenol increased the expression of hypertrophic marker; B-type natriuretic peptide (BNP), CYP genes; CYP1A1, CYP1B1, CYP2J3 as well as the gene encoding the soluble epoxide hydrolase, Ephx2 in a concentration-dependent manner for both time-points 24 and 48hs. No significant changes were observed for CYP2B1, CYP2C11, and CYP4F1. Furthermore, 11, 12-EET attenuated the increased expression of BNP, CYP1A1, and Ephx2 induced by isoproterenol treatment.
Conclusion: Isoproterenol induced cardiac hypertrophy and modulated CYP450 expression in H9c2 cells. In addition, isoproterenol increased the expression of Ephx2, the enzymes which may lead to a consequent decrease in the cardioprotective metabolites, EETs. 11, 12-EET poses a protective effect against isoproterenol-induced cardiac hypertrophy in H9c2 cells.
Acknowledgements: This work was supported by a grant from CIHR to A.S.O.E.

10. Anti-proliferative Effect of *Lactobacillus* Probiotic Supernatant Substrates in Colorectal Cancer: *In vitro* Study

Imen Kahouli, Catherine Tomaro-Duchesneau, Meenkashi Malhotra, Shyamali Saha, Alaoui-Moulay Jamali and Satya Prakash. Faculty of Medicine. McGill University, Montreal Canada.

Purpose: Colorectal cancer (CRC) is the second leading cause of death worldwide. Implication of probiotic cells has been investigated and their effect against CRC has been evaluated. Particularly, lactic acid bacteria (LAB) have been health benefits in many disease specially CRC in-vivo and in-vitro models. Many Lactobacillus strains were tested for their therapeutic and chemopreventive effects in CRC. Thus, the goal of this study is to evaluate the potential anti-cancer effect of Lactobacillus acidophilus excretory products in CRC. Thus, the effect of the probiotic bacterial excretory products on the proliferation of colon cancer cells was investigated.
Methods: For the preparation of the probiotic substrate, the pellet of live bacterial cells was collected form a 16 hours MRS-bacterial culture. Then, the probiotic products were prepared at a concentration that corresponds to 10^8 probiotic cell/ml. Then, they were added with the cell medium and incubated with colon cancer cells for 72 hours.

	Concentra-tion	Cell Viability (%)	Cell apoptosis (%)
Live probiotic cell supernatant	(1:1)	58±4.84	67±3.52
	(1:2)	27± 8.98	31±6.36
Heat-killed probiotic cell supernatant	(1:1)	32±5.89	41±3.95
	(1:2)	13±3.34	17±3.71

Results: SW cancer cells viability and apoptosis after incubation with probiotic supernatants were as below:
Conclusion: This work suggests that live active Lactobacillus excretory products have potential in inhibiting colon cancer cell proliferation and CRC prevention in general. Further research in identifying molecules, mechanisms of action and animal testing will be needed.

11. Colorectal Cancer Anti-proliferative Potential of *Lactobacillus* Probiotic Excretory Products: *In vitro* Study

Imen Kahouli[1,3], Catherine Tomaro-Duchesneau[1], Meenkashi Malhotra[1], Shyamali Saha [1,2], Alaoui-Moulay Jamali[3] and Satya Prakash[1,3]*.
[1]Departments of Biomedical Engineering, Physiology, and Artificial Cells and Organs Research Center, Faculty of Medicine, McGill University, Montréal, Québec, Canada; [2]Faculty of Dentistry, McGill University, Montréal, Québec, Canada; [3]Department of Experimental Medicine, McGill University, Montréal, Québec, Canada.

Purpose: Colorectal cancer (CRC) is the second leading cause of death worldwide. Implication of probiotic cells has been investigated and their effect against CRC has been evaluated. Particularly, lactic acid bacteria (LAB) have been health benefits in many disease specially CRC in-vivo and in-vitro models. Many Lactobacillus strains were tested for their therapeutic and chemopreventive effects in CRC. Thus, the goal of this study is to evaluate the potential anti-cancer effect of Lactobacillus acidophilus excretory products in CRC.
Methods: The effect of the probiotic bacterial excretory products on the proliferation of colon cancer cells was investigated. For the preparation of the probiotic substrate, the pellet of live bacterial cells was collected form a 16 hours MRS-bacterial culture. Then, the probiotic products were prepared at a concentration that corresponds to 108 probiotic cell/ml. Then, they were added with the cell medium and incubated with colon cancer cells for 72 hours.
Results: Results showed the probiotic supernatant extracted from active live bacterial reduced SW-480 cell viability up to 58 % compared to the control group. Cell apoptosis was also investigated and showed an increase up to 67% in treated cells. In addition, the supernatant extracted from dead probiotic cells reduced SW-480 cell viability up to 32 % and increased apoptosis up to 41%.
Conclusion: Live active Lactobacillus excretory products have potential in inhibiting colon cancer cell proliferation and CRC prevention in general. Further research in identifying molecules, mechanisms of action and animal testing will be needed.
Acknowledgements: The authors would like to acknowledge Canadian Institute of Health Research (CIHR) grant (MOP: 936041) to Dr. S. Prakash and FRSQ Doctoral award to Meenakshi Malhotra.

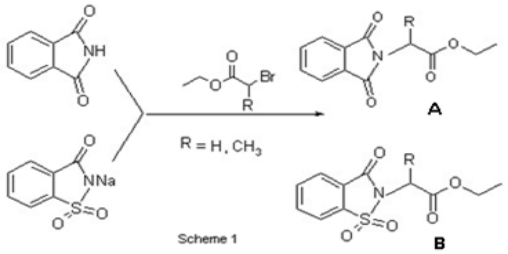
Pharmaceutical & Analytical Chemistry

12. Synthesis of Saccharin and Phthalimide Derivatives as Chelators

Fereshteh Pourmorad, Mohammadali Ebrahimzadeh, Fatemeh Hosseinikhah Choshali, and Niloofar Miri Ahangari. Mazandaran University of Medical Sciences, Pharmaceutical Sciences Research Center, Facualty of Pharmacy, Sari, Iran.

Purpose: Zinc (Zn), copper (Cu) and iron (Fe) are essential minerals that are required for a variety of biomolecules to maintain the normal structure, function, and proliferation of cells. Abnormal metabolism of heavy metals can lead to several chronic pathogenesis. In this study, some compounds with chelating activity have been synthesized. The chelaing acivity of the compounds was also investigated.
Methods: Acetic acid ethyl ester and propionic acid ethyl ester derivatives of saccharin and phthalimide were synthesized by the reaction of the starting materials (Saccharin and phthalimide)with alkyl bromoacetates such as methyl bromoacetate and ethyl bromopropionate under the reflux condition and using the DMF as solvent (Scheme 1). After work-up the obtained products (A and B) were evaluated as chelators by UV method using EDTA as a standard. A and B were refluxed with hydroxylamine hydrochloride in ethanol and then the products isolated. The desired compounds were purified by crystallization by ethanol. The UV spectra of ester products in the absence and presence of EDTA were obtained in order to the analysis of chelating activity with Fe II and III, Cu and Al. All

products were determined by analytical methods (NMR and IR).



Scheme 1

Results: The reactions' yields obtained 50 to 90%. The UV spectra of ester products in the absence and presence of EDTA were obtained in order to the analysis of chelating activity with Fe II and III, Cu and Al. In presence of EDTA the reaction of complex production returned back to previous state which suggests less chelating power of the compound. The compounds showed less chelating activity than EDTA as standard.
Conclusion: Overall, Phthalimide and saccharin derivatives had the ability to chelate Fe III more efficient than that of other metals.

13. Understanding the Interactions of Surfactants with Lipid Membranes Shines Light on their Diverse Applications in Pharmaceutical Industries

Mozhgan Nazari, Dew Das, and Heiko Heerklotz. University of Toronto, Leslie Dan Faculty of Pharmacy

Surfactants are amphiphilic compounds that are divided into two main categories: synthetic detergents and biosurfactants. Exploring the world of surfactants either as individual molecules or their interactions with living systems like cell membranes has amazed and challenged pharmaceutical scientists for centuries. Surfactants have important and diverse applications in pharmaceutical industries; they are used as excipients or active agents. Diverse applications of surfactants can be explained by their very different mechanisms that they are using in their interactions with cell membranes.
Purpose: To study the interactions of surfactants with lipid membranes, to understand the mechanisms that are used by surfactants to perturb the membranes, to classify surfactants based on these mechanisms and to relate them with their pharmaceutical applications according to their characteristics.
Methods: The techniques that are used to study these interactions are time-resolved fluorescence spectroscopy, isothermal titration calorimetry, dynamic light scattering, and zeta potential.
Results and Conclusion: In this study, we propose a classification of surfactants into those that are homogeneously disordering versus heterogeneously perturbing lipid membranes. Typical synthetic detergents such as $C_{12}EO_8$, octyl glucoside, SDS, and lauryl maltoside were identified as homogeneously disordering by the limiting fluorescence anisotropy, r_∞, of several DPH derivatives reaching a characteristic, low level at the onset of solubilisation. The biosurfactants like surfactin, fengycin, iturin, digitonin, and lyso-PC along with the synthetic CHAPS belong to another class that initiates membrane lysis without critical disordering the whole membrane. They disrupt the membrane locally due to a spontaneous segregation from the lipid and/or an ordering effect that induces packing defects. This may account for enhanced activity, selectivity, and mutual synergism of antimicrobial biosurfactants. They should also be prone to form partially demixed, asymmetric micelles (or bicelles) with a relatively lipid- (and sterol?) rich core surrounding a solubilized protein. Zeta potential measurements of liposomes exposed to surfactin show strong peptide-peptide interactions already at 5 mol% in the membrane. These measurements also show that surfactin is protonated and not fully charged at pH 7.4; therefore, it is membrane permeant.

14. Enhanced Auger Electron Radioimmunotherapy of Breast Cancer using Trastuzumab Modified with Metal Chelating Polymers for Complexing Indium-111

Ghislaine Ngo Ndjock Mbong, Yijie Lu, Peng Liu, Mitchell A. Winnik and Raymond M. Reilly, Department of Pharmaceutical Sciences, Department of Chemistry, University of Toronto, Toronto, Ontario.

Purpose: We have been studying Auger electron radioimmunotherapy of human epidermal growth factor 2 (HER2) positive breast cancer (BC) using trastuzumab (Herceptin®) derivatized with diethylenetriaminepentaacetic acid (DTPA) chelators for labeling with Indium-111 (^{111}In) and modified with nuclear localizing sequence (NLS)

peptides [^{111}In-NLS-DTPA-trastuzumab]. ^{111}In-NLS-DTPA-trastuzumab targets HER2+ human BC cells and its nuclear importation causes DNA double strand breaks by the Auger electrons emitted by ^{111}In. The survival of high expression (1×10^6 receptors/cells) HER2+ BC cells exposed to the radioimmunotherapeutic agent was less than 10%. However, low expression (5×10^4 receptors/cells) HER2+ BC cells were insensitive with a survival of 90%. A proposed explanation for this insensitivity was that the achieved specific radioactivity (amount of radioactivity per mass) was low (1 MBq/μg). In order to increase the potency of ^{111}In-labeled-trastuzumab, we conjugated the monoclonal antibody to a metal chelating polymer (MCP) harboring multiple DTPA for complexing ^{111}In: ^{111}In-MCP-trastuzumab.

Methods: The MCP with a polyglutamide backbone, harboring 29 DTPA groups and a hydrazide reactive group was linked to sodium meta periodate oxidized carbohydrates on the Fc domain of trastuzumab. The immunoconjugate was purified by ultrafiltration, labeled with ^{111}In and characterized by instant thin layer chromatography (ITLC) and size-exclusion HPLC interfaced with UV and flow scintillation radioactivity detectors. HER2 immunoreactivity of the immunoconjugate was evaluated by measuring its k_{on} and k_{off} rates to HER2 extracellular domain (HER2-ECD) coated on a gold sensor chip in surface plasmon resonance (SPR) studies. The effective concentration (EC_{50}) needed to displace half the specific binding of the immunoconjugate was obtained in a competitive binding assay on HER2+ SK-BR-3 human BC cells. The specific activity was assessed by ^{111}In-labeling decreasing masses of the immunoconjugate with the same radioactivity (9.25 MBq).

Results: The MCP was successfully conjugated to trastuzumab as shown by the overlay of the UV and radioactive signals on SEC-HPLC. The immunoconjugate binds to HER2-ECD with high affinity (K_D = 0.123±0.019 nM) and 10.95±1.47nM of trastuzumab were required to displace the binding of the immunoconjugate to SK-BR-3 cells. The radiochemical purity was greater than 98%. ^{111}In-MCP-trastuzumab was labeled to a high specific activity (7.4MBq/μg) using a low mass (1μg). This represents a specific radioactivity that is almost 8 fold higher than previously achieved.

Conclusion: We constructed a new radioimmunoconjugate ^{111}In-MCP-trastuzumab that exhibited preserved HER2 immunoreactivity. This radioimmunoconjugate can be labeled with up to 8-fold higher specific radioactivity than ^{111}In-NLS-DTPA-trastuzumab.

15. Design, Synthesis and Biological Evaluation of Phenyl 4-(2-oxo-3-alkylimidazolidin-1-yl)benzenesulfonates, A Novel Class of Antineoplasic Agents Highly Selective Towards Estrogen-dependent Human Breast Cancer Cells

Sébastien Fortin[1,2], Jacques Lacroix[1], Marie-France Côté[1], Xavier Charest-Morin[1], Sophie Parent[3], Éric Asselin[3] and René C. Gaudreault[1,4]. [1]Centre de recherche C.H.U.Q., QC; [2]Faculté de Pharmacie, Université Laval, QC; [3]Université du Québec à Trois-Rivières, Trois-Rivières, QC; [4]Faculté de Médecine, Université Laval, QC, Canada.

Purpose: Breast cancer is the most frequently diagnosed cancer and the second cause of cancer death in women in Canada. Despite the scientific advances of the past 75 years, there are no treatments that are completely effective against that disease. In this context, new treatments improving survival and quality of life are intensively sought. We recently found a new family of potent antimicrotubule agents termed as phenyl 4-(2-oxoimidazolidin-1-yl)-benzenesulfonates (PIB-SOs, exemplified by compound **1**) exhibiting GI_{50} in the low nanomolar range and showing potent antitumoral and antiangiogenic potency in chick chorioallantoic membrane tumor assays (CAM assays). The crystallographic structure of the □,□-tubulin heterodimer, the basic structural unit of microtubules, evidenced an hydrophobic pocket enclosing the phenylimidazolidin-2-one moiety of PIB-SOs, that is an important haptophoric unit for the cytocidal activity of PIB-SOs. In the aim of improving the interactions between PIB-SOs and that hydrophobic pocket on □,□-tubulin, we have modified the phenylimidazolidin-2-one moiety by addition of lower alkyl groups onto the free NH group leading to the preparation of phenyl 4-(2-oxo-3-alkylimidazolidin-1-yl)benzenesulfonates (PAIB-SOs, exemplified by compound **2**).

Methods: Dozens of PAIB-SOs were prepared, characterized and biologically evaluated for their antiproliferative activity. The most potent compounds were assessed for their ability to inhibit the cell cycle progression and to interact with the estrogen and progesterone receptors. The toxicity of PAIB-SOs was also assessed on chick embryos.

Results: Several PAIB-SOs exhibited antiproliferative activities in nanomolar range on estrogen-dependant (ER^+) MCF7 breast carcinoma cells whereas they exhibited antiproliferative activities in micromolar range on other cell lines and notably on estrogen-independant (ER^-) MDA-MB-231 human breast carcinoma, HT-29 colon carcinoma and M21 skin melanoma cells showing a selectivity ratio up to 250 fold for the ER^+ MCF7 cells.

# compounds	GI_{50} (nM)[a]				Selectivity ratio (ER^-/ER^+)
	MCF7 (ER^+)	MDA-MB-231 (ER^-)	HT-29	M21	MDA-MB-231/ MCF7
1	5.0	9.4	4.0	4.0	1.9
2	49	13000	5600	3900	265

[a]GI_{50} is expressed as the concentration of drug inhibiting cell growth by 50%.

PAIB-SOs arrested the cell cycle progression in G_2/M phase, do not show affinity for the estrogen and progesterone receptors and do not show toxicity on chick embryos.

Conclusion. PAIB-SOs are highly selective towards ER^+ MCF7 breast carcinoma cells through an unidentified mechanism of action. The low toxicity of PAIB-SOs on chick embryos suggests that these compounds might be a promising new class of anticancer agents targeting estrogen-dependent human breast cancers.

16. Doxorubicin Conjugated at 16α-position of Estrogen for Site-specific Treatment of Estrogen-receptor Positive Breast Cancer. Design, Synthesis and Cytocidal Activity

Sébastien Fortin, Pijus Saha, Valérie Leblanc, Éric Asselin and Gervais Bérubé. Département de Chimie-Biologie, Université du Québec à Trois-Rivières, Trois-Rivières, Canada

Purpose: Doxorubicin (DOX) is an important medicine for the treatment of breast carcinomas, which are the most frequently diagnosed and the most lethal cancers in women worldwide. However, serious toxic effects such as cardiomyopathy and congestive heart failure are impeding its clinical use. To improve the efficacy and to decrease the cardiac toxicity of DOX, we have assessed a new approach to covalently bind DOX to estrogen to deliver selectively the drug to estrogen receptor-positive (ER^+) cancer tissues. However, literature precedent show that conjugation of anthracyclines to estrogen was performed either at position 3 or 17 of estrogen. Those approaches are not ideal since the hydroxyl groups on positions 3 and 17 are important for receptor binding affinity. In the present study, we have designed, prepared and evaluated *in vitro* the first estrogen-doxorubicin conjugates at position 16α of estrogen (E-DOXs, **8a-d**).

Methods: DOX was conjugated to position 16α of the estradiol carrier via an alkylamide linking arm having 3, 5, 7 or 9 carbon atoms, respectively. E-DOXs were prepared from estrone using a seven-step procedure to afford the desired conjugates in low to moderate yields. The cytocidal activity of E-DOXs was assessed on HT-29 human colon carcinoma, M21 human skin melanoma together with ER^+ MCF7 and ER^- MDA-MB-231 human breast carcinoma cell lines.

Results: The antiproliferative activity of E-DOX **8a** on ER^+ MCF7 and HT-29 cells is in the micromolar range whereas it is mainly inactive on M21 and ER^- MDA-MB-231 cells, thus showing selectivity of this compound (> 3.5 fold) for ER^+ MCF7 vs. ER^- MDA-MB-231. E-DOX **8b-d** derivatives are mainly inactive on all cancer cell lines tested so far. These results show that a short alkylamide linking arm (< 5 carbon atoms) is required for significant and selective antiproliferative activity.

Conclusion: This study suggests that the nature of the anticancer drug conjugated to DOX together with the nature and the chain length of the alkylamide linking arm play important roles in the antiproliferative activity presumably through a synergy between the physicochemical properties and steric hindrance of the conjugates on their affinity for the ER α. Finally, the selectivity of E-DOX **8a** toward ER^+ MCF7 cells indicates that this compound might be useful for site specific treatment of ER^+ breast cancers and the design of more potent derivatives.

17. A Simple and Specific HPLC Assay of Hydroxysafflor Yellow A (HSYA) for Pharmacokinetic Studies in Rats

Haijun Li[1,3], Peicheng Zhang[2]and Pollen K.F. Yeung[1]. [1*]College of Pharmacy, Faculty of Health Professions, Dalhousie University, Halifax, Nova Scotia, Canada; [2]Institute of Materia Medica,

Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China; [3]Institute of Frontier Medical Science, Jilin University, Changchun City, Jilin, China

Purpose: To develop simple and specific HPLC assay for hydroxysafflor yellow A (HSYA) which is a main water soluble component in safflower of the Traditional Chinese Medicine for pharmacokinetic studies.
Methods: HSYA was isolated and purified from *Carthamus tinctorius L.* The HPLC system consisted of a solvent delivery module (Beckman model 114, Berkeley, CA, USA), a 3μm 110A 150 x 3.0mm i.d. C_{18} reversed phase analytical column with a 5μm 4.0 x 3.0mm i.d. C_{18} reversed phase cartridge guard column (Security Guard Cartridges, Phenomenex, Torrance, CA, USA), a Shimadzu SPD-VIS (SPD-20A) spectrophotometric detector (Man-Tech Assoc. Inc., Guelph, ON, Canada), and an Agilent Model 3395 Integrator (Santa Clara, CA, USA). The mobile phase was made up of 0.005 M KH_2PO_4 (pH 5.5): acetonitrile: methanol (92:3:5). The system was operated isocratically at ambient temperature with a flow rate of 0.5 mL/min, and UV wavelength at 254 nm, and an operating pressure of ca. 2.2 – 2.5 kpsi. Extraction of HSYA and the internal standard (IS) 2-phenoxypropionic acid (PPA) from plasma was achieved by protein precipitation using methanol. The extract was dried at 55°C and the residue was stored at -20°C until analysis. The assay was validated for sensitivity, precision, linearity and specificity for pharmacokinetic study in rats (n = 6).
Results: Under these conditions, the retention times of HSYA and the IS were 19 and 11 min, respectively, and recoveries were > 85%. Standard curves using 50 μL of plasma sample were linear from 0.2 to 20 μg/mL, with regression coefficient (r^2) = 0.99 or greater. Sensitivities based on absolute injection were 0.5 ng and 1 ng on column for HSYA and IS, respectively. The intra-assay variations were < 10%, and inter-assay variations were < 20%. Plasma maximum concentration and 6 hours after 10 mg/kg of HSYA given by subcutaneous injection was 10.4 ± 6.5 and 0.13 ± 0.10 μg/mL, respectively.
Conclusion: The described simple HPLC assay had adequate sensitivity and specificity to study pharmacokinetics of HSYA in rats (Supported in part by a studentship from Chinese Scholarship Council to Haijun Li).

Pharmacokinetics & Pharmacodynamics

18. Adenosine 5'-monophosphate Concentration in Red Blood Cell as Potential Surrogate Biomarker for Cardiovascular Toxicity in Rats *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 potential of adenosine 5'-monophosphate (AMP) concentration in red blood cell (RBC) as surrogate biomarker for cardiovascular toxicity *in vivo*.
Methods: Sprague Dawley (SD) rats with a carotid artery catheter weighing between 250 and 300g were used. 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. RBC concentrations of adenine nucleotides and hemodynamic 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 died had significantly greater increase of the AMP concentration than those surviving ones.
Conclusion: Isoproterenol induced significant

mortality and traumatic change of cardiovascular hemodynamic. RBC concentrations of AMP were significantly higher in the dying rats and may be used as surrogate biomarkers for cardiovascular toxicities in preclinical study (Supported in part by CIHR, Nova Scotia Health Research Foundation and Dalhousie Pharmacy Endowment Foundation).

19. Optimizing Periconceptional and Prenatal Folic Acid Supplementation: Steady-state Red Blood Cell and Plasma Folate Levels Achieved with 5mg vs. 1.1mg Folic Acid in Prenatal Multivitamin Supplements among Pregnant Women

Mahvash Shere[1,2], Bhushan Kapur[1,2], Deborah O'Connor[1,2], Gideon Koren[1,2]. [1]University of Toronto, [2]The Hospital for Sick Children

Background: Folic acid supplementation before and during pregnancy reduces the risk of neural tube defects (NTDs). Maximal protection against neural tube defects is achieved through maternal red blood cell (RBC) folate concentrations of 900nmol/L or greater. Although previous studies have investigated folate status in non-pregnant women of childbearing age, steady-state folate pharmacokinetics have never been evaluated in the population of pregnant women to inform healthcare recommendations for the timing of prenatal multivitamin supplementation.
Purpose: To compare the steady-state periconceptional and gestational RBC and plasma folate levels in pregnant women who supplement daily with prenatal multivitamins containing 1.1mg vs. 5mg folic acid.
Methods: Eight women, between 18-45 years of age, who were early in pregnancy or trying to conceive, and were not previously taking folic acid-containing supplements, were enrolled in this open-label, 2-arm, randomized clinical trial after obtaining informed consent. Women with a previous history of neural tube defects or women taking folate antagonists were excluded from the study. Participants were randomly assigned to take either 1.1mg or 5mg of folic acid-containing prenatal multivitamins daily till 30 weeks gestational age. Plasma and RBC folate levels were measured at baseline, and at 6, 12 and 30 weeks of gestation using a competitive-binding receptor assay.
Results: No significant difference was observed between the baseline RBC concentrations of the two groups (baseline was 2849 ±143nmol/L and 2840 ±230nmol/L in the 1.1mg and 5mg groups respectively). However, differences were observed in RBC concentrations between the groups at 6, 12, and 30 weeks gestation: RBC folate concentrations by 30 weeks gestation were 4149 ±321nmol/L in the 1.1mg vs. 6175 ±394nmol/L in the 5mg group.
Conclusion: The use of 5mg folic acid produced and maintained higher blood folate concentrations compared with 1.1mg folic acid, thus rendering greater protection against neural tube defects.

20. The Effect of Inflammation on Expression of β_1, β_2, and β_3-adrenoreceptors in Rat Heart

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

Purpose: Inflammation reduces pharmacological response to β-adrenoreceptor (β-AR) blockers such as propranolol even in the presence of high concentrations. This decrease in potency has been shown due to be caused by downregulation of β_1-AR. Recently, however, we have shown that pre-adjuvant arthritis (Pre-AA) fails to decrease responsiveness to nebivolol, a third generation β_1- and β_2-blocker with NO-generating property via β_3-AR agonistic activity. The aim of this study was to investigate the effect of reduced expression of β_1.AR on response to nebivolol.
Method: We studied expression of β_1, β_2 and β_3-AR in two groups of male Sprague-Dawley rats (n=4 each), Control and Inflamed. On day 0, rats received single injections of 0.2 mL of either *Mycobacterium butyricum* in squalene (Inflamed) or 0.9% normal saline (Control). On day 15, when pre-adjuvant arthritis was confirmed, rats were anesthetized and incisions were made; hearts collected and immediately frozen in liquid nitrogen and stored at -80º C until analyzed. Western immunoblot was used to determine the protein levels of the β_1, β_2 and β_3 subunits of β-AR in the rat heart. β-actin was used as a loading control.
Results: As we expected, the relative density of β_1-AR protein was significantly reduced in inflamed animals as compared with control rats (control: inflamed; 2.27±0.55 vs. 1.45±0.31, p=0.035) while that of β_2-AR and β_3-AR were not significantly influenced by inflammation (control: inflamed; β_2-AR: 0.9±0.02 vs. 0.89±0.13 and β_3-AR: 2.5±0.38 vs. 2.12±0.25).
Conclusion: The lack of effect of inflammation on

response to nebivolol appears to be due to the drug's nitric oxide donating property via β_3 and β_2-AR stimulation as under our experimental conditions, the level of β_2- and β_3-AR remains unaffected by inflammation despite the observed down-regulation of β_1-AR.

21. Potential Role of RAS System Enzymes in NSAIDs Induced Cardiovascular Changes

Waheed Asghar, Ali Aghazadeh-Habashi, Fakhreddin Jamali. University of Alberta, Edmonton AB, Canada

Purpose: Non-steroidal anti-inflammatory drugs (NSAIDs) are used for the management of arthritis and other rheumatoid disorders but their use is restricted due to their adverse effects on gastrointestinal, renal and cardiovascular system. Angiotensin converting enzymes (ACE, ACE2) being one component of Renin Angioten System (RAS) are involved in the regulation of blood pressure and electrolyte homeostasis, through balanced production of their respective peptides (ANG_{II}: vasoconstricting, ANG_{1-7}: vasodilating). In inflammation the constitutive balance between ACE and ACE2 protein is disrupted as observed in the rat heart. NSAIDs can also adversely influence the cardiac function through multiple pathways specifically RAS. Thus NSAIDs when given in inflammatory conditions can worsen the already compromised vascular homeostasis. In order to discover the influence of selected NSAIDs (rofecoxib and meloxicam) on RAS enzymes we looked for the concentration of their active peptides. This is to confirm that an imbalance in ACE and ACE2 protein upon NSAIDs exposure translates into activity and contribute towards their cardiovascular effects.
Methods: Sprague Dawley rats (n=20) were divided into 4groups namely control, inflamed(vehicle) and inflamed treated (rofecoxib 20mg/kg, meloxicam 6mg/kg). The inflamed group received 0.2ml of 50mg/ml of Mycobacterium butyricum in squalene in tail base. On day 12, rats in inflamed group started receiving their respective NSAIDs or vehicle. After 7 days rats were euthanized and their organs were harvested and stored (-80C) until analyzed through ELISA.
Results: Inflammation reduced ANG_{1-7} concentration in the plasma (0.22± 0.07ng/ml) and heart (0.29±0.04ng/gm), compared to control plasma (0.88± 0.04ng/ml) and heart (0.22±0.09 ng/gm). Rofecoxib caused an increase in plasma (9.11±2.55ng/ml) and heart (1.72±0.72ng/gm) so do meloxicam in the plasma (8.56±2.63ng/ml) but not in the heart (0.35±0.03ng/gm). Inflammation resulted into a significant increase in ANG_{II} in plasma (4.66±1.15 ng/ml) and heart (0.42±0.20ng/gm), compared to control rats plasma (0.37±0.12ng/ml) and hearts (0.18±0.04 ng/gm). Rofecoxib caused a further increase in plasma (13.43±3.47gm/ml) heart (1.13±0.19ng/ml) so do meloxicam in plasma (5.38±1.12ng/ml) and heart (0.15±0.06ng/gm).
Conclusion: The activity of ACE and ACE2 enzymes is influence by inflammation and NSAIDs through their effects on RAS. This results into an overall increase in the concentration of ANG_{II} and ANG_{1-7} peptides. But ANG_{II}/ANG_{1-7} ratio (plasma, heart) is much higher for roefcoxib (1.65, 0.88) compared to meloxicam (0.83, 0.44). Thus prevalence of more ANG_{II} over ANG_{1-7} may suggests a dysfunction of the RAS in rofecoxib treated rats, that may contribute to dysregulation of vascular tone and resulting into more cardiovascular consequence.

22. Effect of Glucosamine on Renin-angiotensin System under Inflammatory Condition

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

Purpose: Inflammation is one of the main contributing factors for cardiovascular complications in rheumatoid arthritis (RA). It is associated with the activation of the renin–angiotensin system (RAS). The main components of the RAS are angiotensin (Ang) II and Ang (1-7) that are produced by the angiotensin converting enzyme (ACE) and ACE2, respectively. Ang II is a powerful vasoconstrictor, while Ang (1-7) counteracts the effects of Ang II as a vasodilatator. A balance between ACE and ACE2 is needed to regulate blood pressure, fluid, and electrolyte homeostasis. Inflammation causes downregulation of ACE2 in heart of rats with adjuvant arthritis (AA), hence, disturbs the ACE2-ACE balance. Glucosamine (GlcN) possesses anti-inflammatory properties. We have hypothesized that GlcN is able to counteract the effects of inflammation on the expression of ACE2 and restore the ACE2-ACE balance.

Methods: Male Sprague-Dawley rats were randomly assigned to six groups (n=4-5): Control-placebo, INF-placebo, INF-GlcN (20, 40, 80, 160). On day zero control and INF animals received, saline or 0.2ml Mycobacterium Butyricum in Squalene (50 mg/mL) as tail base injection, respectively. The INF-GlcN and placebo groups were received daily oral dose of 20, 40, 80 or 160 mg/kg GlcN or water, respectively. On day 16 placebo groups and on day 22 INF-GlcN groups were euthanized and their plasma and heart were harvested, kept at -80° C until analyzed for nitrite and protein expression, respectively.
Results: Serum nitrite concentration was significantly elevated due to inflammation but GlcN treatment restored the levels. Inflammation significantly reduced expression of ACE2 protein and ACE2/ACE ratio but ACE protein expression was not affected significantly. GlcN treatment showed a dose-dependent response on increasing ACE2 protein expression and restoring the ACE2/ACE ratio.
Conclusion: The anti-inflammatory effects of GlcN cause re-establishment of the disturbed ACE2-ACE balance due to inflammation. This suggests a cardioprotective potential for GlcN in inflammatory conditions such as rheumatoid arthritis.

23. The Poloxamer 407 Model of Hyperlipidemia; Dose Responsiveness, Duration of Hyperlipidemia, and Effect on Adipokine and Cytokine Levels

Hetal R. Chaudhary and Dion R. Brocks. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada.

Purpose: Intraperitoneal (IP) injection of a non-ionic surfactant, poloxamer 407 (P407), is known to induce significant levels of serum lipoprotein in rodents. Little is known about the relationship between the doses of P407 and lipid levels and how long the hyperlipidemia (HL) persists after the agent is administered. In the present study, we investigated P407 induced HL in rat.
Methods: P407 was dissolved in cold normal saline (0.13 g/mL) and injected IP into rats at two different doses (0.5 g/kg and 1 g/kg). Blood samples were collected from each rat through tail vein at 0, 36, 60, 84, 108, 132, 156, 180 and 282 h after P407 injection. Serum was collected and the levels of total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL) cholesterol were measured using commercially available kits. Serum samples were analyzed for adiponectin, leptin and TNF-α level using commercially available ELISA kits. **Results**: P407 increased serum cholesterol, triglyceride and HDL cholesterol and the effect was dose dependent. The maximum increase in lipids was observed at 36 h, which remained elevated for up to ~132 h and ~180 h for the 0.5 and 1 g/kg doses, respectively. Log-cholesterol and triglyceride levels displayed linear decreases after time of maximal lipid concentrations (C_{max}). The mean baseline concentration of lipids (C_0), C_{max}, $AUC_{0\text{-}282\ h}$ and the half-life ($t_{1/2}$) to return to normal values, were:

Para-meters	Total Cholesterol	Triglyceride	Total Cholesterol	Triglyceride
	0.5 g/kg		1 g/kg	
C_0, mg/dL	64.4±13.3	172.7±39.7	53.2±16.2	207.9±79.1
C_{max}, mg/dL	1271±181	7005 ±1175	2390±272	9973±1004
$t_{1/2}$, h	21.9	13.8	26.4	16.5
AUC, mg×h/L	8121±672	32790±3405	19596± 6042	78954± 25506

In addition to changes in lipids, P407 significantly increased serum leptin and decreased the serum adiponectin concentrations. Nevertheless, no significant effect was observed on the proinflammatory cytokine TNF-α.
Conclusion: P407 induces hyperlipidemia with the maximum increase in lipid levels occurring at 36 h. After P407 the HL effect is sustained for up to 8 days. P407 also alters adipokine in an expected manner, but did not seem to affect cytokine concentrations.

24. An Alternative Liquid Chromatography-Mass Spectroscopy Method for the Quantification of Azithromycin in Human Plasma; Utility in Determination of Azithromycin Pharmacokinetics in Obese Patients

Mohamed Ben-Eltriki[1], Vishwa Somayaji[1], Raj S. Padwal[1,2] and Dion R. Brocks[1,2]. [1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta; [2]Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Canada.

Purpose: To develop a simple, selective and rapid liquid chromatographic mass spectrometric (LC-MS)

assay for the quantification of azithromycin in human plasma.

Methods: Azithromycin and imipramine (as internal standard, I.S.) were extracted from human plasma (0.5 mL) using one step liquid–liquid extraction with 4 mL of diethyl ether under alkaline conditions by adding 20 µL of 10 M ammonium hydroxide. Chromatographic separation of drug and I.S was performed using Kinetex XB-C18 column 2 .6 µm (2.1 × 50 mm) at room temperature. A mobile phase consisting of methanol and water (75:25, v/v) mixture containing 0.2% ammonium hydroxide and 0.1% ammonium formate was pumped at an isocratic flow rate of 0.2 mL/min. The mass spectrometer was operated in positive ion mode with selected ion recording (SIR) acquisition mode. The ions utilized for quantification of azithromycin and I.S. were m/z 749.6 (M+H) + and m/z 591.4 (fragment) for azithromycin, and 281.1 m/z for I.S, with retention times of 6.9 and 3.4 min, respectively.

Results: The calibration curves were linear (>0.999) in the concentration ranges of 10 to 1000 ng/mL. The mean absolute recoveries for 50 and 500 ng/mL azithromycin and 1 µg/ mL I.S were 75.9, 77.5% and 94.7 %, respectively.The percentage coefficient of variation and mean error of the inter- and intra-day validations were <11%, 6%, respectively. The lower limits of quantification were 10 ng/mL.

Conclusion: The present method was successfully applied to determine azithromycin pharmacokinetic parameters during a pharmacokinetic study conducted on 14 female obese volunteers. Our results indicated that overall, the pharmacokinetics of azithromycin were comparable in our obese volunteers to literature data from lean subjects.

25. Contribution of Intestinal ABC and SLC Drug Transporters to the Intestinal Absorption and Drug-drug Interactions of the HIV-protease Inhibitor, Atazanavir (ATV)

Olena Kis[1], Sharon Walmsley[2], Reina Bendayan[1]. [1]Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto; [2]Division of Clinical Investigation & Human Physiology, Toronto General Research Institute/ Department of Medicine, Faculty of Medicine, University of Toronto

Purpose: Several interactions can occur between antiretroviral drugs (ARVs) in the intestine. The objective of this study was to investigate the role of drug carriers in facilitating or restricting the intestinal absorption of the HIV protease inhibitor (PI), atazanavir (ATV), and their potential contribution to ATV drug-drug interactions.

Methods: ATV intestinal permeability was measured *in vitro*, across Caco-2 monolayers and *in situ*, by single-pass perfusion of rat intestine, in the absence or presence of ARVs and specific transporter inhibitors: PSC833 and GF120918 for Pgp; FTC for BCRP; BSP, MK571 and rifamycin SV for MRPs/OATPs; E3S, pravastatin and probenecid for OATPs/OATs; PAH for OATs; and procainamide, MPP and TEA for OCTs. The expression of drug transporters and enzymes was analyzed by real-time qPCR and immunoblotting.

Results: ATV uptake by Caco-2 cells was stimulated by acidic extracellular pH and susceptible to inhibition by several OATP family inhibitors (estrone-3-sulphate, rifamycin, probenecid, pravastatin), suggesting that ATV cellular uptake is carrier-mediated. Efflux of ATV was potently inhibited by Pgp inhibitors (PSC833, GF120918) and several PIs (darunavir, lopinavir, saquinavir). Some ARVs, such as ritonavir and tenofovir disoproxil fumarate (TDF), inhibited both uptake and efflux of ATV at clinically-relevant concentrations. Absorption and secretion of ATV across Caco-2 cell monolayers were concentration-dependent, and ATV permeability (P_{app}) from basolateral (BL) to apical (AP) compartment was 11.7-fold higher than its P_{app} (AP-BL). This efflux ratio was reduced to 1.5 in the presence of Pgp inhibitors, i.e., PSC833 and GF120918, as well as the coadministered PI, ritonavir. Pgp inhibition also significantly increased ATV effective permeability (P_{eff}) *in situ* (78 and 79% in rat jejunum and ileum, respectively, $p < 0.0001$), demonstrating that Pgp restricts ATV intestinal absorption. Furthermore, TDF significantly lowered ATV intestinal permeability by 28% in the *in situ* perfusion model, mirroring the interaction observed clinically, suggesting that intestinal epithelium may contribute to the observed ATV-TDF clinical interactions.

Conclusions: PIs can interact with several intestinal influx and efflux transporters at clinically relevant concentrations. Since these carriers can transport many drugs, including statins and other ARVs, these drug transporters may contribute to clinically significant drug-drug interactions in the intestine.

This study was funded by CANFAR; Olena Kis was a recipient of an NSERC and OHTN scholarships and was recently awarded a CIHR-CGS studentship

award; Drs Reina Bendayan and Sharon Walmsley are recipients of the OHTN Career Scientist award.

26. Fetal Lopinavir Exposure - The Impact of PXR and ABC Drug Transporters

Sarabjit Gahir and Micheline Piquette-Miller. Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada

Purpose: Lopinavir, a widely used drug for managing HIV positive pregnancies, is believed to be a substrate of drug transporters including Mdr1 and Mrp2. The role of major placental drug transporters (Mdr1, Mrp2 and Bcrp) in determining the fetal exposure to Lopinavir was assessed. Since we have previously shown the placental expression of key drug transporters to be linked to Pregnane X Receptor (PXR) genotype, we also examined the impact of fetal PXR genotype on trans-placental transport of Lopinavir.
Methods: Utilizing PXR -/- mice, we generated PXR +/- mothers bearing PXR +/+, PXR +/- and PXR -/- fetuses. Since the expression of placental transporters is determined by fetal genotype, a wide range of transporter expression was obtained within the same mother in our animal model. Animals were dosed with 10mg/kg Lopinavir i.v. and plasma and fetal levels of the drug were measured by LC-MS/MS 30 mins post injection. The placental transporter levels were estimated using quantitative real time PCR. All fetal units were genotyped for PXR by visualizing the PCR products on a 2% agarose gel.
Results: Mdr1a and Mrp2 were found to be involved in determining the fetal exposure to Lopinavir. A significant, linear correlation was observed between fetal Lopinavir exposure and placental Mdr1a expression. With an increasing level of placental Mdr1a, the fetal accumulation of Lopinavir was reduced dramatically. Similarly, a significant, but weaker, linear correlation was observed between expression level of placental Mrp2 and fetal accumulation of Lopinavir. The Lopinavir exposure in PXR +/+ units was approximately 2 fold higher than PXR -/- fetal units. No correlation was observed between Bcrp expression and Lopinavir fetal exposure.
Conclusion: Our findings demonstrate that placental Mdr1a and Mrp2 expression levels are major determinants of fetal exposure to Lopinavir. Additionally, Bcrp has no discernible impact on the trans-placental trafficking of the drug. Interestingly, while the PXR genotype of the fetal unit was shown to have an impact on the fetal accumulation of Lopinavir, this effect was only evident at the extreme spectrum of our animal models; i.e. in the PXR -/- and PXR +/+ units. Furthermore, neither the PXR genotype of the fetal units, nor the transporter expression at the placenta, seemed to have any impact on intra-uterine development.

27. Transferring from Clopidogrel Loading Dose to Prasugrel Loading Dose in Acute Coronary Syndrome Patients: The TRIPLET Trial*

Jean G Diodati,[1] Anthony Fung,[2] Jorge F. Saucedo,[3] Abdurrahman Oguzhan,[4] Efrain Gaxiola,[5] Tracy E Cardillo,[6] Mark B. Effron,[6] Harold N. Fisher,[7] Carsten Henneges,[8] Dominick J. Angiolillo[9] on behalf of the TRIPLET investigators. [1]Hôpital du Sacré-Coeur de Montréal, Montreal, QC; [2]Vancouver General Hospital, Vancouver, BC; [3]University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; [4]Erciyes University, Kayseri, Turkey; [5]Jardines Hospital de Especialidades, Zapopan, Jalisco, México; [6]Eli Lilly and Company, Indianapolis, IN, USA; [7]Eli Lilly and Company, Toronto, Canada; [8]Eli Lilly and Company, Bad Homburg, Germany; [9] University of Florida College of Medicine-Jacksonville, Jacksonville, FL, USA

Purpose: Patients (pts) with acute coronary syndrome (ACS) are often loaded with clopidogrel (C) prior to coronary angiography (angio). Prasugrel (P) may be preferred once coronary anatomy is known and PCI is planned. While there are data describing the pharmacodynamic (PD) effect when switching from C maintenance dose (MD) to P (with or without a P loading dose (LD)), no PD data are available on the effect of switching directly from C LD to P LD.
Methods: A randomized double-blind study in ACS pts with intent to undergo PCI was performed to determine the PD response of 3 thienopyridine LD strategies: (1) placebo (PBO) LD followed by P 60 mg LD compared to (2) C 600 mg LD followed by P 60 mg LD and (3) C 600 mg LD followed by P 30 mg LD. All pts received the C/PBO LD prior to diagnostic angio, but only pts who were to undergo PCI received a P LD. Pts loaded with P were given P 10 mg MD daily if PCI was completed. Platelet reactivity was measured as P2Y12 reaction units

(PRU) with the VerifyNow P2Y12 assay prior to P LD (up to 24 h after C/PBO LD), and at 2, 6, 24, and 72 h post P LD. The primary objective was to evaluate the mean difference in PRU, measured at 6 h following a P 60 mg LD, in ACS pts undergoing PCI who were pretreated with C 600 mg LD vs. PBO LD. A linear mixed model was used to calculate LS mean estimates.

Results: 282 pts received the initial C/PBO LD (safety population). After diagnostic angio, 149 pts underwent PCI, received P LD, and had at least one evaluable PRU measurement post P LD. At 6 h following P 60 mg LD, pts who received a 600 mg C LD had no statistically significant difference in PRU (Table) when treated with P (60 or 30 mg) compared with a P 60 mg LD alone. In the safety population, there were 3 haemorrhagic adverse events in PBO/P60 mg LD, 3 in the C 600 mg/P 60 mg and 6 in the C 600 mg/P 30 mg.

Table: VerifyNow P2Y12 PRU at 6 Hours Post P LD

Treatment (N patients/ N evaluable PRU samples)	PRU			
	Median (Min/Max)	LS Mean (SE)	LS mean difference (upper/lower CI)	p-value
[1] placebo and pras 60 mg (52/43)	10.0 (0.0, 376.0)	57.86 (11.86)		
[2] clop 600 mg and pras 60 mg (47/38)	9.0 (0.0, 324.0)	35.61 (12.36)	22.24 (-10.98, 55.47)	0.188[a]
[3] clop 600 mg and pras 30 mg (50/45)	15.0 (0.0, 317.0)	53.92 (11.74)	3.93 (-28.20, 36.07)	0.809[b]

Difference [a][1] vs [2] and [b][1] vs [3]

Conclusion: Patients with ACS who undergo a PCI after receiving C LD and P LD (either 60 mg or 30 mg) achieve a similar level of platelet reactivity to P 60 mg LD alone.

*Encore presentation of abstract presented at the EuroPCR congress, Paris France, May 15-18, 2012.

28. Regulatory Affairs: Update on Clinical Pharmacogenomics (PGx) – FDA Draft Guidance (2011) Document: Premarketing Evaluation in Early Phase Studies: Overview and Perspectives

E. Jane Mitchell, PhD, RAC. OptumInsight™, Dundas, ON; Jane.Mitchell@optum.com

FDA's 2011 draft guidance on clinical development in early phase studies will be presented from the perspective of regulatory science in drug product development and drug lifecycle and where further direction is still required. FDA illustrates the value of PGx in development by three case histories of drugs already having PGx/Biomarker information in their Prescribing Information (PI), namely Abacavir, Clopidogrel, and Warfarin. Currently, there are more than 100 drug labels approved by FDA with biomarker information to aid physicians in dosing, e.g., DOSING TABLE, or avoiding adverse events, lack of efficacy, e.g., WARNING, BLACK BOX WARNING and improve outcomes in disease management and patient care. In early studies, FDA indicates e.g., in healthy volunteers, clinical PGx -PK and -PD should be done with prospective DNA analysis with information with known ADME variants to assist in identification of outliers; *in vitro* studies suggest polymorphic pathways and pro-drugs activated therein should be characterized appropriately with subjects having generic variants; dose/response studies may be stratified by e.g., genotype to aid interpretation. This adds to earlier PGx guidance documents, since 2005, from FDA, EMA, Health Canada largely concentrated on definitions, background, concepts, format and content of submissions including Voluntary Exploratory Drug Submissions (VXDS), the process for meetings, including joint meetings with FDA/EMA and described the agency working groups. In this 2011 FDA draft guidance, DNA sampling is discussed in broad terms of its being prospective, having consent, and retained, if new PGx issues arise. However, it does not address the bioethics, legal and informed consent issues arising from future circumstances that would allow these samples to be used retrospectively or if so how this could this impact the patient? How informed would informed consent really be here? Are we anticipating a new different ethical and legal process? Would there be a central ethics committee guiding these decisions and who would it be composed of? FDA as well as other stakeholders will need to better understand and work through these points as this PGx area of regulatory science moves ahead.

Drug Delivery & Pharmaceutical Technology

29. Evaluation of Cellular Uptake and Intracellular Trafficking as Determining Factors of Gene Expression for Amino Acid-substituted Gemini Surfactant-based DNA Nanoparticles

Jagbir Singh[1], Deborah Michel[1], Jackson M. Chitanda[2], Ronald E. Verrall[2], Ildiko Badea[1]. [1]Drug Design and Discovery Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, [2]Department of Chemistry, University of Saskatchewan

Purpose: Gene transfer using non-viral vectors offers a non-immunogenic and safe method of gene delivery. Cellular uptake and intracellular trafficking of the nanoparticles can impact on the transfection efficiency of these vectors. Therefore, understanding the physicochemical properties that may influence the cellular uptake and the intracellular trafficking can aid the design of more efficient non-viral gene delivery systems. Recently, we developed novel amino acid-substituted gemini surfactants that showed higher transfection efficiency than their parent compound. In this study, we evaluated the mechanism of cellular uptake of the plasmid/gemini surfactant/helper lipid nanoparticles and their effect on the transfection efficiency.
Methods: Gemini surfactants were formulated with a model plasmid (pGT.IFN-GFP) in the presence of a helper lipid (DOPE) creating P/G/L nanoparticles at a charge ratio of 1:10 of P/G. Cell toxicity of endocytic inhibitors (genistein, filipin III, methyl-β-cyclodextrin, chlorpromazine hydrochloride, wortmannin) alone and in presence of P/G/L nanoparticles was evaluated using MTT assay. The plasmid and the helper lipid were fluorescently tagged to track the nanoparticles inside the cells, using confocal laser scanning microscopy (CLSM). Particle size and buffering capacity of the P/G/L nanoparticles were measured by pH titration from basic to acidic pH. DNA binding properties of the gemini surfactants were evaluated by dye exclusion assay in presence of polyanions (heparin).
Results: Clathrin-mediated and caveolae-mediated uptake were found to be equally contributing to cellular internalization of both P/12-7NH-12/L (parent gemini surfactant) and P/12-7NGK-12/L (amino acid-substituted gemini surfactant) nanoparticles. Dye exclusion assay and pH-titration of the nanoparticles suggested that high buffering capacity, pH-dependent increase in particle size and balanced DNA binding properties may be contributing to a more efficient endosomal escape of P/12-7NGK-12/L compared to the P/12-7NH-12/L nanoparticles, leading to higher gene expression.
Conclusion: Amino-acid substitution in the spacer of gemini surfactant did not alter the cellular uptake pathway, showing similar pattern to the unsubstituted parent gemini surfactant. Glycyl-lysine substitution in the gemini spacer improved buffering capacity and imparted a pH-dependent increase of particle size. This property conferred to the P/12-7NGK-12/L nanoparticles the ability to escape efficiently from clathrin-mediated endosomes. Balanced binding properties (protection and release) of the 12-7NGK-12 in the presence of polyanions could contribute to the facile release of the nanoparticles internalized via caveolae-mediated uptake. A more efficient endosomal escape of the P/12-7NGK-12/L nanoparticles lead to higher gene expression compared to the parent gemini surfactant.

30. Assessment of Novel Oral Lipid-based Formulations of Amphotericin B Using an *in vitro* Lipolysis Model

Fady Ibrahim [1], Pavel Gershkovich [1], Olena Sivak[1], Ellen K. Wasan[1,2], Kishor M. Wasan[1]. [1]Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC, Canada; [2]School of Health Sciences, British Columbia Institute of Technology, Burnaby, BC, Canada

Purpose: The purpose of this study was to investigate the intraluminal processing of novel oral lipid-based formulations of amphotericin B using an *in vitro* lipolysis model.
Methods: Amphotericin B (AmB) was formulated in three lipid-based formulations consisting of different lipid components; iCo-009, iCo-010 and iCo-011. The concentration of AmB in the three formulations was 4.2-4.3 mg/ml. Various lipid loads (0.25, 0.5,1 and 2 g) were digested using the lipolysis model. At the end of lipolysis, samples were withdrawn from the digestion medium and were subjected to ultracentrifugation. The concentration of AmB in the separated layers was assessed using a validated HPLC method.
Results: The duration of lipolysis was comparable

among the three formulations except for 2 g load of iCo-009 which had a significantly longer lipolysis than iCo-010 and iCo-011. The lipid components of iCo-009 experienced lower extent of lipolysis as compared to other formulations. Amphotericin B concentration in the aqueous phases was the highest from iCo-010 which also had the lowest sediment recovery. Amphotericin B levels in the undigested lipid layers were comparable between iCo-009 and iCo-010 and were higher than with iCo-011.
Conclusion: Taken together the observation that iCo-010 had the highest aqueous micellar solubilization and the lowest sediment recovery of AmB among the tested formulations, these results can be used to interpret and predict the *in vivo* performance of AmB lipid-based formulations in future studies.

31. In Vivo and in Vitro Tissue Repair Due to Hialuronan Jelly

Loida Oruña[1,2], Gabriel Coto[2,3], Radu Nanau[4,5], Manuela Neuman[3,4]. [1]State Center of Quality and Drug Control, [2]Latin American Faculty of Medicine, Havana City, Cuba; [3]Histoterapy Placentary Center, Havana City, [4]Department of Clinical Pharmacology and Toxicology, University of Toronto Canada; [5]In Vitro Drug Safety and Biotechnology, MaRS, Toronto, ON, Canada.

Substantial evidence strongly suggests that oxidative stress plays a role in the pathogenesis of wound healing. Hyaluronidan jelly, hyaluronic acid (HA) is a glycos-amino-glycan synthesized from umbilical cord. Previously we show that in an in vivo model hyaluronidan is able to enhanced wound healing. In vitro, in human epithelial cells damaged by ethanol exposure, hyaluronidan also induced cell repair.
Purpose: The present study aimed to link the experiments *in vivo* and *in vitro* utilizing concentration of 2%, 4% and 8 %) HA.
Material and Methods: Excision wounds have been inflicted in 120 Sprague Dawley rats and HA was used to help healing. In vitro we treated A431 epidermoid skin cells and mice fibroblast with two concentrations of ethanol for 24 hours. We employed HA in three concentration levels to determine its efficacy in the treatment
Results: *In vitro* ethanol was toxic to cells in a dose dependent manner and increased the percentage of cells undergoing apoptosis. Treatment of cells with 50 and 100 mM ethanol increased release of the proinflammatory cytokine tumor necrosis factor-alpha (TNF-α) into culture medium. HA in the 2% and 4% concentrations reduced inflammation both in human A431 epidermoid skin cells and in mouse fibroblast cell line. In vivo studies, that employed 4% HA-jelly showed the higher epitelization of the wound as well as epithelial migration for histology results and dermal reconstitution as determined by alpha amino-nitrogen (4.32 mg/area), hydroxiproline (10.3 mg/area).
Conclusions: Both *in vivo* and *in vitro* studies demonstrate the efficacy of the use of hyaluronidan in wound-repair, thus suggesting the possible clinical use of the product.

32. Pharmaceutical Manufacturing by Foam Granulation in a Twin Screw Extruder

Michael R. Thompson[1], Bo Mu[1], Paul J. Sheskey[2]. [1]Department of Chemical Engineering, McMaster University, Canada; [2]The Dow Chemical Company, Midland, MI.

Purpose: Improving our understanding of foam granulation as a new continuous method of manufacturing pharmaceutical solids, as the approach avoids over-saturation during granule nucleation.
Methods: Granulation of α-lactose monohydrate (FlowLac® 100, Meggle Pharma) was conducted with 6% or 11% (w/w) aqueous foamed binder in a twin screw extruder (ZSE 27HP, Leistritz) at rates of 10-30 kg/h and screw speeds of 220-320 RPM. Three hydroxypropyl-methylcellulose binders (METHOCEL™ E-PLV series; Dow Chemical, Midland, MI) were prepared as 4% (w/w) aqueous solutions to give differing viscosities (7-35 mPa-s). Foam quality was varied from 75-95% using a mechanical foam generator. Granules were characterized for particle size and fracture strength. Properties of the aqueous foams were characterized for their free drainage time and shear stability to understand their wetting behavior in the granulation process.
Results: Higher liquid drainage rates were found in foams with lower foam quality and made with lower viscosity binders. Foam stability was found to be lower at lower shear rates and with higher foam quality, both states corresponding to higher shear stresses in the flowing media. Foamed binder addition resulted in uniformly wetted excipient filler even at low liquid content (i.e. 6%), avoiding the

surging and segregation observed when liquids were directly added. Observations found the foam partially collapsed on contact with the moving bed of lactose (first nucleation stage) while the remaining foam was carried over the lactose as a separate phase till it collapsed downstream (second stage). Final particle growth showed stronger dependency on operating factors (screw speed and flow rate) compared to foam properties, indicating a mechanical dependency for the nucleation mechanism. For all conditions with 11% liquid, less than 10% of the original particles remained (i.e. <250 μm), with the majority of granules (~60%) being larger than 1000 μm. At 6% liquid, less than 24-50% of the original particles remained, depending on conditions. The lowest viscosity binder generally produced larger granules with higher fracture strength.

Conclusions: The study revealed a two-stage nucleation process that was strongly dependent on the mechanical forces inside the extruder. Lower viscosity binders produced foams with higher drainage rates and higher shear stability, which were subsequently linked to producing larger granules with higher fracture strength.

33. Molecular Mechanism of Doxorubicin (DOX) Loading onto and Release from Poly(methacrylic) Acid Grafted Starch Nanoparticles

Gaurav Raval, Alireza Shalviri, Helen Fan, Xiao Yu Wu and Heiko Heerklotz. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto

Purpose: A thorough understanding of molecular interactions between drug and polymeric carrier is important for rational design of drug delivery systems. The aim of this study was therefore to investigate molecular interactions of anti-cancer drug doxorubicin (DOX) with negatively charged poly(methacrylic acid) grafted starch (PMAA-g-St) and to interpret drug loading and release data.

Methods: Effect of pH and sodium chloride (NaCl) on DOX/PMAA-g-St interactions and DOX dimer dissociation process was investigated using Isothermal Titration Calorimetery (ITC). Drug release studies were carried out at 37 °C in aqueous buffer solutions of different pH. Zeta potential and Z-average size of DOX/ PMAA-g-St nanoparticle complex were measured using dynamic light scattering (DLS) in titration experiments. Fluorescence intensities were monitored by fluorescence spectroscopy as a function of DOX concentration and by titrating PMAA-g-St into DOX solutions.

Results: ITC results showed that DOX bound very strongly to negatively charged carboxylic acid ($-COO^-$) on PMAA-g-St (with estimated K_D <1 μM) governed predominantly by electrostatics. Zeta potential, ITC and fluorescence spectroscopy studies suggested that DOX could dimerize both in aqueous solution and in polymer-bound state. With increasing pH, the drug loading capacity of PMAA-g-St nanoparticles increased, whereas the drug release from PMAA-g-St decreased. With increase in NaCl concentration, the drug loading capacity of PMAA-g-St decreased due to charge shielding effect. Fluorescence experiments showed that in presence of PMAA-g-St, DOX dimers broke into monomers and then each subsequent monomer bound to accessible binding sites. By comparing ITC and DLS results, it was found that DOX first bound with high affinity into the inner core of PMAA-g-St as suggested by constant zeta potential value(~ -40mV) and decrease in Z-average size due to compaction of PMAA-g-St co-polymer. Once all the high affinity sites were occupied, DOX monomer bound to outer surface leading to charge neutralization (zeta ~ 0 mV) at [DOX]/[COOH] ratio of 1:1. At [DOX]/[COOH] >1, the zeta potential of the nanoparticles became positive (+30 mV) and aggregation of the nanoparticles occurred. A model for multispecies equilibrium was developed to estimate K_D value of DOX dimer in polymer bound state.

Conclusion: This work has elucidated and quantified DOX binding with PMAA-g-St nanoparticles and DOX self-dimerization. Combination of techniques such as ITC, DLS, fluorescence spectroscopy and the multispecies equilibrium model enables us to interpret quantitatively the data of drug loading onto and release from polymeric nanoparticles.

34. Hot Melt Granulation Using a Twin Screw Extruder with Model Drugs (Ibuprofen and Caffeine) in a PEG Matrix

Sharleen Weatherley[1], Michael R. Thompson[1], Paul J. Sheskey[2]. [1]Department of Chemical Engineering, McMaster University, Canada; [2]The Dow Chemical Company, Midland, MI.

Purpose: To explore the effect of continuous hot melt granulation on the characteristics of manufactured tablets containing various amounts of lactose, polyethylene glycol, and active pharmaceutical ingredient (ibuprofen or caffeine).
Methods: Hot melt granulation of 65-78.5% (w/w) α-lactose monohydrate (FlowLac® 100, Meggle Pharma), 6.5-20% (w/w) polyethylene glycol (PEG 8000 or PEG 3350; Dow Chemical, Midland, MI), and 0% or 15% (w/w) of either ibuprofen USP (Spectrum Laboratory) or caffeine (Sigma-Aldrich), was conducted in a twin screw extruder (ZSE 27HP, Leistritz), at a rate of 5 kg/h, screw speed of 220 RPM, and barrel temperature of 100°C. Granules were characterized for particle size. Classified granules within the range of 250-850 μm were pressed into tablets and characterized for fracture strength, with the resulting tablet being subjected to a USP <711> dissolution test, with samples taken at 15, 30, 45, and 60 minutes without replacement, and an "infinity" sample taken at 75 minutes after vigorous stirring.
Results: During the granulation process, the temperature of the exiting granules varied between 100-118°C. The motor load varied between 3-9 A, with the caffeine trials having a consistently higher load than the ibuprofen trials (6-9 A versus 3-6 A). After granulation, between 34-55% of the particles were within the desired particle range of 250-850 μm. The amount of fines (<250 μm) varied from 2-24% and the amount of chunks (>850 μm) varied from 24-63%. The characteristic fracture strengths of the granules varied between 15953-80244 MPa, with almost all of the fracture strengths of granules containing active ingredient being higher than granules that contained none. For the dissolution tests, there appeared to be no difference between samples prepared with ibuprofen or caffeine, or samples prepared with PEG 8000 or PEG 3350, after 75 minutes. All samples dissolved between 76-96% of the total amount of active ingredient. In general, higher dissolution values were obtained from the extruded samples than from hand-blended samples.
Conclusions: The study revealed that ibuprofen was easier to process than caffeine and the granulation process created particles within the desired size range. The fracture strength of granules with active ingredient was higher than those without. Granulation appeared to increase the dissolution of active ingredient.

35. Effect of Drug-Polymer Interactions and Polymer Composition on Drug Permeability and Partition in Eudragit RL/RS 30 D Membranes

Kuan Chen, Michael Chu, Stephanie Kwan, Xiao Yu Wu. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Canada

Purpose: Eudragit RL 30 D (RL) and Eudragit RS 30 D (RS) are acrylic polymers widely used in the pharmaceutical industry for coating solid dosage forms. With high and low permeability, respectively, RL and RS polymers can be blended in varying ratios to achieve different release profiles. Although the polymers are well characterized, little is known on how drug properties influence their permeability and partition in the polymers of various RL/RS ratios. This study investigates how drug properties and polymer composition affect thermodynamic and kinetic parameters of drug.
Methods: Polymer membranes with different RL:RS ratios were prepared by casting polymer dispersions of RL and RS. Diltiazem HCl, ibuprofen Na, and theophylline were used respectively as cationic, anionic and neutral model drugs. Drug partition between RL/RS blend membranes and aqueous medium was determined by using the equilibrium sorption method. Drug permeability through thin polymer membranes was measured with side-by-side diffusion cell. In addition, the Hildebrand solubility parameter was calculated for the polymers and the drugs using the Van Krevelen group contribution method. For each term in the Hildebrand solubility parameter, the squared difference between the polymer and the drug was calculated to evaluate drug-polymer interactions.
Results: Theophylline had much higher permeability than diltiazem and ibuprofen, with ibuprofen being the lowest. Both diltiazem and ibuprofen showed lag time in the permeation curves, which may be attributed to drug-polymer electrostatic interactions. Ibuprofen showed the highest partition into the polymers, likely due to electrostatic complexation between the anionic drug and the cationic polymers. Diltiazem also showed partition into the polymers, but theophylline showed negligible partition. In terms of the Hildebrand solubility parameter, both diltiazem and ibuprofen had similar squared difference terms that were smaller than theophylline's, indicating that both diltiazem and ibuprofen had stronger interactions

with the Eudragit polymers than theophylline. The solubility parameter results correlated well with the lower permeability and higher partition coefficients observed in both dilitazem and ibuprofen as compared to theophylline.
Conclusion: Drug-polymer interactions have a large effect on drug partition and permeability in RL and RS polymer membranes and ultimately the release profiles of the formulations. The thermodynamic and kinetic parameters determined in this work are important for computer simulation of drug release profiles and computer-aided design of controlled release dosage forms.

36. Lysine-Functionalized Nanodiamonds: Synthesis, Physicochemical Characterization and Potential as Gene Delivery Agents

Randeep Kaur[1], Jackson M. Chitanda[2], Deborah Michel[1], Ronald E. Verrall[2], Ildiko Badea[1]. [1]Drug Design and Discovery Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, [2]Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

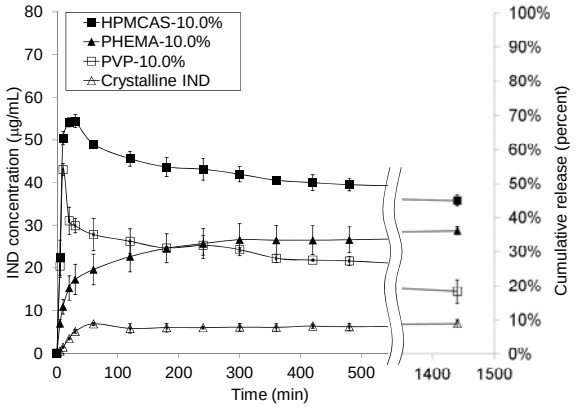
Purpose: Detonation nanodiamonds (NDs) are carbon-based nano-materials that are emerging as bioimaging agents and promising tools for delivering biochemical moieties into cells. Nano-size (4-5 nm), alterable surface chemistry, fluorescence and biocompatibility are some of the characteristic properties of NDs which promote their use in biomedical sciences. However, the tendency of diamond particles to self-aggregate in liquid media leads to formation of micron-sized aggregates, hampering their applicability in biological systems. Here, we describe the covalent functionalization of NDs by using lysine with the aim of achieving positively charged disaggregated particles that could serve as potential carriers for genetic materials.
Methods: The NDs were oxidized and covalently functionalized with lysine through a 3-carbon length linker. Raman spectroscopy, FTIR, zeta potential, thermogravimetric, size and atomic force microscopic analyses were conducted to characterize lysine-modified NDs. The binding properties of functionalized diamonds with plasmid DNA (pDNA) and small interfering RNA (siRNA) were investigated by gel-electrophoresis assay, and size and zeta potential measurements.
Results: NDs were successfully functionalized with the lysine-linker producing a surface loading of 1.7 mmol g^{-1} of ND. The functionalization approach resulted in considerable reduction in the aggregate size of NDs from 1280 nm to 20 nm and they formed highly stable aqueous dispersions with zeta potential of +49 mV. The lysine-modified NDs showed ability to bind pDNA and siRNA at a calculated lysine/base pair of nucleic acids ratio of, 6/1 and 20/1, respectively. Based upon the physicochemical characterization and binding ratios, we developed a model of "diamoplexes" showing complex formation between the NDs and genetic materials.
Conclusion: Considerable disaggregation of NDs was achieved after their covalent functionalization with lysine and these lysine substituted NDs showed potential to act as carriers for genetic materials.

37. Extent of Solubility Enhancement of Indomethacin Amorphous Solid Dispersions in Crosslinked Poly(2-hydroxyethyl methacrylate) Hydrogels

Dajun D. Sun, Tsu-chi Rob Ju, and Ping I. Lee. University of Toronto, Toronto, Ontario, Canada

Purpose: To evaluate the kinetic solubility profiles and solubility improvement of indomethacin amorphous solid dispersion in insoluble hydrogels versus those in water-soluble polymers under a nonsink dissolution condition.
Methods: The crosslinked poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel beads and amorphous solid dispersions (ASD) of indomethacin (IND) in PHEMA and in water-soluble PVP and HPMCAS at various drug loadings were prepared based on previously published methods. The morphology, crystallinity, Tg, and drug-polymer interactions of the resulting IND-PHEMA ASD systems were examined by SEM, XRD, DSC and FTIR and the drug loading thresholds for the amorphous-to-crystalline transition were identified. The kinetic solubility profiles at various IND drug loadings and PHEMA bead particle sizes were evaluated and compared with those of PVP-IND and HPMCAS-IND systems under a nonsink dissolution condition. Stability studies on IND ASD in PHEMA were carried out according to ICH long-term ($25^{o}C/60\%RH$) and accelerated ($40^{o}C/75\%RH$) conditions.
Results: The threshold of amorphous-to-crystalline transition ASD in water-soluble polymers is

identified to be at approximately 25% IND as compared to that of 34% IND in PHEMA based on the XRD data. The extent of solubility improvement of IND ASD in PHEMA falls between those in PVP and HPMCAS at 10.0% IND loading after 6h and outperforms those in PVP and HPMCAS at 33.0% IND loading after 8h. The IND ASD release from water-soluble polymers exhibits an initial surge of drug dissolution followed by a sharp decline. However, the drug dissolution from a less rapidly released system based on insoluble PHEMA hydrogels shows the absence of such a sudden surge and decline during dissolution. Moreover, IND ASD in PHEMA exhibit consistent physical stability for up to 8 months at IND loadings below 16.7%.



Conclusion: The drug release mechanism of rapid dissolution of IND ASD in water-soluble polymers is completely different from the dissolution of IND ASD in PHEMA hydrogel beads which is governed by a feedback-controlled diffusion thus avoiding an initial surge of drug concentration followed by rapid re-crystallization process in dissolution. Our results demonstrate the potential advantage of ASD of poorly water-soluble drugs in PHEMA as compared with that prepared in conventional water-soluble polymers due to its ability to maintain solubility enhancement for a longer duration at higher IND loadings.

38. MicroSPECT/CT Imaging: Advancing Our Understanding of the Biodistribution of Molecularly Targeted Nanoparticles for Cancer Therapy

Niladri Chattopadhyay[1], Humphrey Fonge[1], Zhongli Cai[1], Deborah Scollard[1], Jean-Philippe Pignol[2,4], Eli Lechtman[2] and Raymond M. Reilly[1,3,5].

[1]Departments of Pharmaceutical Sciences, [2]Medical Biophysics and [3]Medical Imaging, University of Toronto; [4]Sunnybrook Health Sciences Centre; [5]Toronto General Research Institute, University Health Network, Toronto, ON, Canada

Purpose: Gold nanoparticles (AuNPs) represent an important class of nanoparticles being investigated for the detection and treatment of cancers. The major barrier in translating these approaches to clinical use is the extensive trapping of nanoparticles by the liver and spleen leading to poor delivery to the tumour. Here, using microSPECT/CT as an imaging tool, we have looked at enhancing tumour retention and intracellular delivery of AuNPs while reducing liver and spleen exposure by systematic evaluation of the impact of tumour targeting and route of administration on organ distribution of AuNPs.

Methods: To optimize the delivery of AuNPs we have developed a multifunctional AuNP platform targeted against HER-2 and labeled with ^{111}In that allows evaluation of their tumour and normal tissue distribution by microSPECT/CT imaging as well as by ex vivo γ-scintillation counting. Mice bearing subcutaneous HER-2-positive human breast cancer (MDA-MB-361) tumours were injected with HER-2 targeted (Au-Ts) or non-targeted (Au-NTs) AuNP via tail vein (i.v) with or without prior injection of 10 mg/kg $GdCl_3$ (for deactivation of macrophages) and non-specific antibody (rituximab, 1 mg/dose) for blocking of Fc mediated liver uptake or intratumourally.

Results: Upon systemic administration Au-NT had a longer plasma half-life and two-fold higher (2.20 ± 0.23 %ID/g) tumour accumulation in MDA-MB-361 xenograft compared to Au-T (1.23 ± 0.20 %ID/g). Liver exposure of Au-T (1.61 ± 0.51 %ID/g) was significantly lower than Au-NT (4.56 ± 0.82 %ID/g) when given intratumourally. Liver exposure upon intratumoural injection with Au-NTs was comparable to that observed when these AuNPs were administered i.v. with or without macrophage deactivation, suggestive of diffusion from the tumour site. MicroSPECT/CT imaging demonstrates AuNP accumulation only in the right axillary lymph node (ipsilateral to tumour) suggesting drainage of AuNPs via the lymphatic system. Ex-vivo transmission electron microscopy confirms cellular internalization of Au-Ts with little or no internalization of Au-NTs.

Conclusion: This study demonstrates that 30 nm Au-Ts delivered intratumourally are effectively

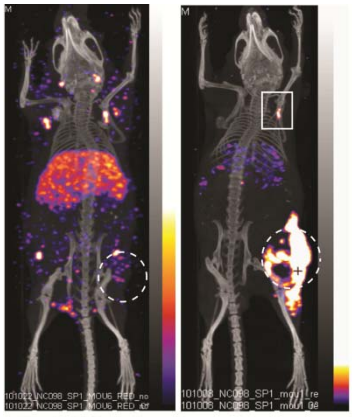


Fig 1. ^{111}In based microSPECT/CT imaging of AuNPs. Circles enclose the tumors and the square encloses the axillary lymph node.

retained (~30% ID/g) with minimal exposure to liver and spleen. Trastuzumab allows efficient internalization and retention of Au-T in HER-2-positive tumour cells. Our findings have important implications for understanding the tumour and normal tissue localization of AuNPs and other targeted and non-targeted nanoparticles administered systemically or locally for treatment of malignancies.

Supported by a Vanier Canada Graduate Scholarship from the Canadian Institute of Health Research and U.S. DOD Breast Cancer Research Program Predoctoral Fellowship (W81XWH-08-1-0519, P00002) to NC and by an operating grant ((grant 019374) from the Canadian Breast Cancer Research Alliance to RMR and JPP.

39. Novel Bispecific Radioimmunoconjugates for SPECT/CT Imaging of HER2 and HER3 Receptors in HER2 Overexpressing Breast Cancer

Eva Razumienko, Deborah Scollard, Raymond Reilly. Department of Pharmaceutical Sciences, University of Toronto, Toronto, Canada

Purpose: In order to initiate downsteam signalling pathways leading to aberrant growth and reproduction of tumour cells, HER2 must heterodimerize with another member of the EGFR family, such as HER3. Our objective was to construct novel bsICs capable of binding both HER2 and HER3 and labeled with ^{111}In for SPECT imaging of both receptors, and for potential future imaging of HER2/HER3 heterodimerization in breast cancer.

Methods: bsICs were composed of trastuzumab (Herceptin) Fab fragments recognizing HER2 linked to a 7.5 kDa HER3-binding peptide of heregulin-β1 (HRG). Fab fragments were produced by digestion of trastuzumab IgG with papain. These were then modified with sulfo-SMCC or related analogues harbouring various length polyethyleneglycol (PEG) spacers (SM-PEG_{12}-NHS and SM-PEG_{24}-NHS) to introduce maleimide groups for cross-linking to HRG that was thiolated with Traut's reagent. bsICs were derivatized with DTPA for labeling with ^{111}In. The ability to independently bind HER2 or HER3 was determined in competition assays using ^{111}In-bsICs against unlabeled Fab or HRG on cells expressing HER2, HER3 or both receptors. The tumour and normal tissue uptake of the bsRICs was then examined in mice bearing BC xenografts that expressed HER2, HER3 or both receptors, and blocking with Fab and HRG performed to determine specificity for the target receptors.

Results: Conjugation of Fab to HRG was confirmed by Western blot and size-exclusion HPLC. bsICs were labeled with ^{111}In to a radiochemical purity of 98.9 ± 0.2% (specific activity 375 ± 14 MBq/mg). Insertion of a PEG spacer between Fab and HRG was required for displacement of binding of ^{111}In-bsICs to HER3 but not to HER2, with the most efficient displacement observed with the PEG_{24} spacer. ^{111}In-bsRICs localized into tumours expressing HER2, HER3, with the best uptake observed at 48 hrs p.i. Uptake was decreased when tumors were pretreated with an excess of Fab in HER2+ xenografts and with HRG in HER3+ xenografts, confirming that the probes could bind specifically to each receptor *in vivo*.

Conclusion: ^{111}In-bsICs composed of trastuzumab Fab and pHRG bound specifically to HER2 and HER3 when an appropriate length PEG spacer was inserted between these two binding moieties, both *in vitro* and *in vivo*.

40. Complete Tumour Regression by a Thermosensitive Liposomal Formulation of Gemcitabine

Jonathan P. May, Mark J. Ernsting, Tatsuaki Tagami, Shyh-Dar Li*. Drug Delivery and Formulation Group, Medicinal Chemistry Platform, MaRS Centre, South Tower, Toronto, Ontario.

Purpose: Pancreatic cancer is often diagnosed after the tumour has become inoperable and locally advanced, leading to poor prognosis (survival time <2 years). Outcomes are only marginally improved

with the standard of care chemotherapy, gemcitabine (GEM). GEM is a nucleoside analogue whose mode of action halts DNA synthesis, leading to cell death. However, GEM has relatively poor pharmacokinetics (PK) and is rapidly metabolized in vivo. Our hypothesis was to encapsulate GEM in a thermosensitive liposome (TSL) to protect the drug from enzymes and reduce clearance rates, thereby allowing more drug to reach the target. A thermosensitive approach was chosen in order to trigger burst release of GEM and create high local concentrations of GEM in the tumour vasculature. Here we describe the development of HaT-GEM (**H**eat **a**ctivated cyto**T**oxic containing gemcitabine), a TSL with improved GEM release properties. Drug release, PK, biodistribution and efficacy has been studied relative to a benchmark standard, lysolipid-thermosensitive-liposome (LTSL), and free GEM.

Methods: GEM liposomes were prepared from DPPC/Brij78 (96:4) (HaT) or DPPC/MSPC/DSPE-PEG (84:10:4) (LTSL) using the thin-film hydration method and passive drug loading technique. Drug release profiles were studied at a range of temperatures and time points. Samples were quenched on ice, spin filtered to isolate released GEM, and GEM concentration was measured by LC (λ = 260 nm). For in vivo studies liposomes were prepared and dosed at 20 mg/kg; free GEM was dosed at 120 mg/kg (both single doses). All formulations were injected i.v. into mice via the tail vein and studied in experiments for PK, biodistribution and efficacy.

Results: HaT-GEM and LTSL-GEM showed similar physical formulation parameters (size: ~100 nm; PDI: ~ 0.1). Drug release rate was 8- to 28-fold increased for HaT-GEM over LTSL-GEM at mild hyperthermic temperatures (39-42°C). Both formulations were equally stable at 37°C with little drug leakage for at least 30 min. GEM PK was much improved for both liposomal formulations; drug clearance was reduced by 50-fold over free GEM. Biodistribution demonstrated a significant improvement of HaT in GEM delivery to the heated tumour compared to free drug and LTSL, 25- and 7.5-fold, respectively. Finally, one single dose of HaT at 20 mg/kg in combination with local heating completely regressed the tumour in 5 days, whilst LTSL-GEM showed moderate activity and free GEM displayed no efficacy.

Conclusions: This data shows great potential for TSL delivery of GEM for cancer and suggests HaT could potentially improve pancreatic cancer treatment.

41. Formulation Strategies to Optimize the Long Term Stability of Gemini Surfactant-based Lipoplexes used for Gene Therapy

Waleed Mohammed-Saeid[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; [2]Department of Chemistry, University of Saskatchewan.

Purpose: Cationic lipid based gemini surfactants have been extensively studied as non-viral vectors for gene therapy. Clinical applications of cationic lipid/DNA lipoplexes are restricted by their instability in aqueous formulations. In this work, we investigated the influence of the lyophilization process on the essential physiochemical properties of gemini surfactant/pDNA lipoplexes and *in-vitro* transfection. Additionally, we evaluated the feasibility of lyophilization as a technique for preparing a lipoplexes with long term stability.

Method: Diquaternary ammonium gemini surfactant (12-7NH-12) and plasmid DNA encoding interferon-γ were used to prepare gemini surfactant/pDNA lipoplexes. Several excipients (sucrose, trehalose, lactose, polysorbate 80, PEG 1450, PEG 8000, glycerine, and combinations therefore) were evaluated as cryoprotectant agents. In an accelerated stability study, four lyophilized formulations were stored at 25˚C and 40˚C. The formulations were analyzed at one, two and three month periods for physical appearance and physiochemical properties (particle size and zeta potential). In addition, *in vitro* transfection efficiency in COS-7 cell and cytotoxicity were assessed. The effect of lyophilization and storage conditions on gemini surfactant/pDNA complex morphology were evaluated using circular dichroism spectroscopy.

Results: The accelerated stability study showed that both sucrose and trehalose provided the anticipated cryoprotective effect. The transfection efficiency of the lipoplexes increased 2-4 fold compared to fresh formulations upon lyophilization. This effect could be related to the improvement of DNA compaction and lipoplexes morphological changes due to the lyophilization/rehydration processes. The physiochemical properties of the lyophilized formulations (particle size and zeta potential) were maintained throughout the three months study. Upon three month storage, all formulations showed a significant loss of gene transfection activity at both

temperatures. Nevertheless, adequate levels of transfection efficiency were preserved for three formulations after two months storage at 25 ˚C.

Conclusion: In conclusion, lyophilization is a suitable method to preserve the essential physiochemical properties of lipoplexes and to maintain the transfection efficiency. The loss of transfection activity upon storage is most probably due to the conformational changes in the supramolecular structure of the lipoplex as a function of time and temperature, rather than to DNA degradation. Additionally, lyophilization could be used to improve the transfection efficiency of gemini surfactant-based lipoplexes.

42. Probiotics: Bio-therapeutics for the Prevention and Treatment of Dental Caries and Oral Candidiasis

Shyamali Saha[1,2], Catherine Tomaro Duchesneau[1], Meenakshi Malhotra[1], Imen Kahouli[1,3], Maryam Tabrizian[1,2], Satya Prakash[1]. [1]Biomedical Technology and Cell Therapy Research Laboratory, Department of Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, QC,. [2]Faculty of Dentistry, McGill University, Montreal, QC, [3]Department of Experimental Medicine, McGill University, Montreal, QC, Canada.

Purpose: Oral diseases pose major health and economic burdens. Dental caries (DC) and oral candidiasis (OC) are two of these diseases. The primary causative organism of DC and OC is *Streptococcus mutans* and *Candida albicans*, respectively, with *C. albicans* also found in late DC. Current prevention and treatment procedures for DC and OC have important limitations. Preliminary research into probiotics proposes their potential in the inhibition of these two pathogenic microorganisms. The purpose of the presented research is to screen probiotic strains for the inhibition of *S. mutans* ATCC 702062 and *C. albicans* ATCC 11006, responsible for DC and OC.

Methods: Probiotic Lactobacilli were screened (in-vitro) for their ability to prevent and treat DC and OC. An assay was developed to demonstrate the clearance zones, indicating the inhibitory potential of live probiotic cells. *S. mutans* and *C. albicans* were incorporated in molten agar at concentrations of 0.5% (v/v) and 1% (v/v), respectively. Live probiotic cell cultures (100µL) were incorporated in pre-formed wells in agar plates. Following an incubation period of 48 hours at 37°C with 5% CO_2, the plates were evaluated for clearance zones. Dose optimization was evaluated in further experiments, using four different concentrations of probiotic overnight cultures.

Results: *L. reuteri* NCIMB 701359, *L. reuteri* NCIMB 701089, *L. reuteri* NCIMB 11951, *L. reuteri NCIMB* 702656, *L. reuteri* NCIMB 702655, *L. fermentum* NCIMB 5221, *L. fermentum* NCIMB 2797, *L. fermentum* NCIMB 8829, *L. acidophilus* ATCC 314, *L. plantarum* ATCC 14917 and *L. rhamnosus* ATCC 5310 were screened and demonstrated clearance zones for the inhibition of both *S. mutans* and *C. albicans*. The extent of the observed clearance was strain-specific. The clearance was normalized by determining the number of CFU required to observe a 1mm zone of clearance. Among all the screened probiotics, *L. fermentum* NCIMB 2797 required the least number of cells, 1.33 x 10^7 cells for the inhibition of *S. mutans* and 9.07 x 10^6 cells for *C. albicans*.

Conclusion: This research demonstrates that all of the screened probiotic cells could be potentially used as bio-therapeutics for the prevention and treatment of DC and OC. However, *in vitro* and *in vivo* experiments should be performed to evaluate the mechanisms of action of the observed inhibition.

Acknowledgement: The authors would like to acknowledge a Canadian Institute of Health Research (CIHR) grant (MOP 264308) to Dr. S. Prakash and a FRSQ Doctoral award to Meenakshi Malhotra.

43. Modeling Different Dextromethophan Dosage Forms Using Gastro Plus

Muhammad K Sarfraz,[1] M. Alkmkainzi,[1] M. Al-Hallak[1] and R. Löbenberg.[1]*. [1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Physiological based pharmacokinetic in silico modeling has become an integral tool in formulation development. The purpose of this study was to investigate how modeling can assist formulation scientist in developing a controlled release dosage form of a drug, which is sensitive to pharmacogenomic variations. Dextromethophan (DM) was taken as model drug because for this drug extensive (EM) and poor metabolizers (PM) exist.

Methods: A predictive model was generated using various physiological and pharmacokinetic

parameters from literature data. Simulations were performed using a 30 mg immediate release (IR) tablet model with or without co-administration of quinidine (25 mg, 50 mg & 75 mg) as enzyme inhibitor. After validation of the predictive model simulations were performed with a zero order release tablets (F_1), first orders sustained release tablets (F_2), initially release (35% in 30 min) followed by the zero order release (F_3) and initially release (70% in 30 min) followed by a zero order release tablets (F_4).

Results: There was a significant difference between EM and PM in all simulations. The IR showed a fast onset, short t_{max} and highest C_{max}, compared to all other dosage forms in both the EM and PM. The AUC_{0-24} was similar for all dosage forms in EM and PM respectively. The co-administration of quinidine showed a gradual increase in plasma drug concentrations with increasing doses, however the effect was more pronounced in EM than in PM. There was gradual increase in C_{max}, decrease in t_{max} and comparable AUC_{0-24} as formulation release profile altered from F_1, F_2, F_3 and F_4 respectively. The formulations were able to alter the drug plasma profiles but did not impact the difference in the observed drug plasma profiles in EM and PM.

Conclusion: Silico modeling was able to predict the drug plasma profiles of different dosage forms. This can assist the formulation scientist to optimize the release properties of a drug from a dosage form to reach a desired drug plasma profile. However, the study showed that drug delivery can not address the differences between EM and PM which were due to pharmacogenomics variations.

44. Nanoparticles Loaded with Rifampicin and Moxifloxacin Induced Variable Levels of Activation in Murine Alveolar Macrophages

W. Shi[1] M. Sarfraz,[1] W. Roa[3] and R. Löbenberg[1*]. [1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada; [2]Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Pulmonary delivered nanoparticles can be taken up by the alveolar macrophages harboring the mycobacterium tuberculosis (Mtb). The goal of the study was to prepare and characterized rifampicin (RF) and moxifloxacin (MF) loaded gelatin and poly-butylcyanoacrylate (PBCA) nanoparticles (Nps) and to investigate the release of inflammatory markers by macrophages after NP exposure to murine alveolar macrophages.

Methods: MF loaded PBCA NPs were prepared using an emulsion polymerization method. RF and MF loaded gelatin Nps were prepared using a two-step desolvation method. A zeta seizer was used to measure the particle size and zeta potential. Transmission electron microscope (TEM) pictures were used to study the morphology of the Nps. MH-S cells were treated with different concentration of nanoparticles in six well plates. The supernatant was collected at different time intervals and was assayed qualitatively using a mouse cytokine antibody array and quantitatively by Enzyme-linked Immunosorbent assay (ELISA) kits.

Results: The Nps were spherical in shape with particle size range from 150- 300 nm. Quantitative data showed the presence of TNF-α, IL-1β, IL-10, IL-12p40 along with NO and H_2O_2 after interaction with PBCA and gelatin Nps as compared to free drug, blank NPs and lipopolysaccharides (LPS) as positive control. The qualitative results were more pronounced with PBCA than gelatin Nps. The results were independent of Nps size, concentrations and drug loading. NO and TNF-α concentration raised initially followed by a gradual decrease. IL-1β, IL-10, IL-12p40 and H_2O_2 showed no significant difference up to 48 hours as compared to the positive control. MF and RF induced very low inflammatory marker except MF can induce higher TNF-α and NO than LPS and blank Nps. The induced inflammatory markers were influenced by nature of the polymer used; gelatin Nps induced relatively higher IL-1β levels than PBCA.

Conclusion: Nps exposure triggered an increase in certain inflammatory markers, which was independent of the size, concentration, drug loading of the nanoparticles.

45. Retargeting Doxorubicin to the Mitochondria to Evade Multidrug Resistance

Graham Chamberlain and Shana O. Kelley. Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto, Toronto, Canada

Purpose: The advent of new technologies allowing the delivery of cargoes to the mitochondria has meant that this organelle can now be a direct target of therapeutics. This provides a strategy through which existing therapies can be retargeted in order to alter their therapeutic utility. Specifically, multidrug

resistance has diminished the effectiveness of many anticancer therapies. Thus, we examined the potential for targeting doxorubicin to the mitochondria in order to evade those resistance mechanisms, thereby increasing the efficacy of the therapy.

Methods: Doxorubicin was conjugated to a mitochondrial-penetrating peptide (mtDOX) using conventional peptide synthesis methods. mtDOX was compared to doxorubicin to examine subcellular localization, the mechanism of action, and the ability to evade resistance.

Results: mtDOX was found to localize to mitochondria in cells compared to a nuclear localization of the parent compound doxorubicin. mtDOX also maintained the ability to intercalate DNA and inhibit topoisomerase II. A PCR-based assay demonstrated differences in the targeting of DNA lesions resulting from incubation of either mtDOX or doxorubicin. Doxorubicin introduced lesions into the nuclear genome, whereas mtDOX caused lesions in the mitochondrial genome. mtDOX was effective at circumventing numerous resistance mechanisms. In particular, mtDOX was able to circumvent P-glycoprotein mediated resistance. Cytotoxicity, uptake, and efflux of mtDOX remained unchanged by effects of P-glycoprotein pumps.

Conclusions: Retargeting therapies such as doxorubicin to mitochondria provides a novel method in which multidrug resistance can be overcome. Through sequestration of the compound within mitochondria they are rendered inaccessible to efflux pumps, increasing accumulation within the cell. These data suggest that other therapies susceptible to multidrug resistance could benefit from being targeted to the mitochondria to evade efflux.

46. Hyaluronic Acid–tocopheryl Succinate Micelles Containing Rifampicin for Active Targeting to Macrophage

Yuan Gao[a], Muhammad Khan Sarfraz[a], Sophie-Dorothee Clas[b], Wilson Roa[c], Raimar Löbenberg[a,*]. [a]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada; [b]Merck, Basic Pharmaceutical Sciences, West Point, PA, USA; [c]Department of Radiation Oncology, Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canadaf

Purpose: The aim of this study was to develop a new nano-sized drug delivery system for the delivery of rifampicin to macrophages with CD 44-ligand.

Methods: New amphiphilic conjugates were synthesized by chemical conjugation of hydrophobic α-tocopherol succinate (TS) to the hydrophilic hyaluronic acid (HA) with different ratio. Their chemical structure and self-association behavior was investigated using ^{1}H-NMR, IR and dynamic light scattering. Rifampicin-loaded HA-TS micelles (RIF-HA-TS) uptake by alveolar macrophage cells (MH-S) was investigated. In addition, their cytotoxicity was determined using the MTT assay. Further the mechanism of uptake and immunological activities were evaluated.

Results: The HA-TS formed self-aggregating micelles in aqueous medium via a hydrophobic interaction between TS, which were stable for least for 2 weeks at 37 °C. The cell uptake studies demonstrated that the RIF-HA-TS had a significant uptake compared to that of the free RIF solution, with the highest uptake at 12 h. The micelles retained their specific biological recognition of the HA receptor CD44, which is present on the macrophages. The HA-TS micelles were taken up into cells via phagocytosis as well as receptor mediated endocytosis, which is energy-dependent. There was a significant increase of RIF-HA-TS uptake by the E. coli LPS activated MH-S compared to the control. Additionally, the RIF-HA-TS micelles had lower cytotoxicity than the free drug solution. Finally, the cytokine studies demonstrated that RIF-HA-TS induced a higher concentration of Th1 cytokines than the free drug. The blank HA-TS micelles induced cytokines related to Th1 in macrophage as well as LPS activated macrophages. This might be an advantage because Th1 cytokines are essential in the fight against the mycobacterium.

Conclusion: The HA-TS micelles could thus provide a promising stable and effective nano-carrier for the delivery of RIF to macrophages.

47. A New Dissolution Method to Assess the In vitro Release of Nanoparticles

Yuan Gao[a1], Jieyu Zuo[a1], Nadia Bou-Chacra[b], Terezinha de Jesus Andreoli Pinto[b], Sophie-Dorothee Clas[c], Raimar Löbenberg[a,*]. [a]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada; [b]Faculty of Pharmaceutical Sciences, Sao Paulo, Brazil; [c]Merck,

Basic Pharmaceutical Sciences, West Point, PA, USA.

Purpose: The aim of this study was to investigate the *in vitro* release of nanoparticles (NPs) using a new dissolution method which can be performed in a standard dissolution tester.
Methods: Two model drugs, rifampicin (RIF) (hydrophobic) and moxifloxacin (MX) (hydrophilic) were used. Gelatin and poly-butylcyanoacrylate (PBCA) nanoparticles were used as model carriers respectively. The drug dissolution and release kinetics from the loaded NPs were studied over time in different media. The release profiles were fitted to different models using DDSolver, which is an Excel plugin module to assess drug release.
Results: The drug release from NPs was pH dependent. According to the observed data, MX and RIF were released from gelatin NPs by diffusion in the absence of trypsin, a serine protease used to facilitate the *in vitro* hydrolysis of gelatin. The release mechanism was a combination of diffusion and erosion in the presence of trypsin. Furthermore, MX released from PBCA NPs was best described by Fickian diffusion, while RIF was released from PBCA NPs by anomalous diffusion. Our results show that the new dissolution method can discriminate between different *in vitro* release mechanisms.
Conclusion: The new dissolution method is an alternative method to determine drug release from nano-carriers using standard dissolution equipment. The release profiles were more uniform compared to dialysis bags.

48. The Effect of Breast Cancer Cell Targeting Peptide Ligand Density on the Tumor Selective Delivery of Doxorubicin by Stealth Liposomal Formulations

Mostafa Shahin[1], Rania Soudy[1], Kamaljit Kaur[1], 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: The aim of this study was to develop actively targeted stealth liposomal doxorubicin (DOX) based on an engineered breast tumor targeting peptide ligand, p18-4, and assess the effect of p18-4 modification on the selective cytotoxicity and cellular uptake of formulated DOX.
Methods: Liposomes composed of hydrogenated soy phosphatidylcholine (HSPC), cholesterol, distearoylphosphatidylethanolamine–poly(ethylene glycol) (DSPE-PEG), and different molar ratios of DSPE–PEG–p18-4 peptide conjugate were prepared (1.5% and 0.3% peptide/total lipid mole). The in vitro release of DOX from liposomes with and without peptide modification was evaluated in phosphate buffer pH 7.4. The cellular uptake of different DOX liposomal formulations as well as free DOX after 24 h incubation with human cancer MDA-MB-435 and MCF-7 cells and the non-cancerous human fibrocystic mammary cells MCF-10A, and mouse dendritic cells DC was evaluated using flow cytometry. The cytotoxicity of different DOX liposomal formulations and free DOX were evaluated using MTT assay after 24 h incubation and the selectivity index (IC_{50} in non-cancerous cells to the IC_{50} in cancer cells) was calculated.
Results: All p18-4 modified liposomes showed slow release of DOX over 72 h, in a manner similar to that of unmodified liposomes. Compared to unmodified liposomes, liposomes decorated with 1.5 mole % p18-4 showed 12 and 10 folds increase in DOX uptake with MDA-MB-435 and MCF-7 cells, respectively. However, liposomes modified with lower p18-4 peptide density (i.e., 0.3 mole %) showed 2 folds increase in DOX uptake in both cells. Flow cytometry results revealed that p18-4 peptide density significantly affected the cellular uptake of DOX by cancerous cells but it had no significant effect on the uptake of liposomes by non-cancerous cells (MCF-10A and DC cells). Compared to unmodified liposomes, 1.5 mole% p18-4 peptide modified liposomal DOX showed 2.4, 5 folds decrease in IC_{50} in MDA-MB-435 and MCF-7 cells, respectively. On the other hand, modification of liposomal DOX with (0.3 mole %) p18-4 peptide led to 1.6 and 2.2 folds decrease in IC_{50} of DOX in MDA-MB-435 and MCF-7 cells, respectively. Liposomal DOX with (0.3 mole %) p18-4 peptide have shown better selectivity against MDA-MB-435 and MCF-7 cells compared to liposomes decorated with (1.5 mole %) p18-4 peptide.
Conclusion: p18-4 decorated liposomal DOX may be used for active targeting of DOX to breast tumor models. The density of p18-4 peptide on liposomal formulations plays a significant role determining the uptake, cytotoxicity as well as selectivity of formulation against target cells.

49. Tri block Copolymers of Poly(ethylene glycol) and Functionalized Poly(ε-caprolactone): Synthesis and Characterization of their Thermo-responsive Sol-gel Transition

Nazila Safaei Nikouei[1], Mohammadreza Vakili[1] and Afsaneh Lavasanifar[1,2*]. [1]Faculty of Pharmacy and Pharmaceutical Sciences, University of 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-caprolactone) and assess the potential of these ABCs in thermo responsive gel formation through characterization of the sol to gel behavior of their aqueous solutions.
Methods: Tri block copolymers composed of poly(ethylene glycol) (PEG) in the middle and poly(a-benzyl-ε-caprolactone) on sides were synthesized through ring opening polymerization of α-benzyl carboxylate-ε-caprolactone by dihydroxylated PEG in the absence of any catalyst. 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 at different catalyst (activated charcoal) levels and reaction times led to a controlled level of reduction of the PBCL lateral blocks producing poly(α-carboxyl-co-benzyl caboxylate-ε-caprolactone) PCBCL. The percentage of debenzylation of the resultant copolymers was determined by ^{1}H NMR. The sol-gel transition behavior of prepared block copolymers in aqueous media was assessed by inverse flow method at different temperatures in the 8-60°C range.
Results: The degree of debenzylation on the PBCL chain was well controlled under synthetic condition particularly by changes in the catalyst concentration. The presence of carboxylic group on PCBCL chain was shown to introduce thermo sensitivity to the polymeric aqueous solution. In general PCBCL-PEG-PCBCL block copolymers have shown sol-gel-sol transition behavior in a 8-55°C temperature range. The percentage of debenzylation affected the transition temperature and the critical gelation concentration (CGC) of block copolymers. The 44% debenzylated polymer showed a sol-gel transition at 31°C at 7%(W/W) concentration and became sol at 41 °C. Decreasing the debenzylation from 44% reduced the sol-gel transition temperature, while polymer with higher percentage of debenzylation showed higher sol-gel transition temperatures at this polymer concentration.
Conclusion: The results points to a potential of tri block copolymers of PCBCL-*b*-PEG-*b*-PCBCL in the formation of thermo-responsive gels.

50. A Slow Releasing Docetaxel-incorporated Nanoparticle Depletes Cancer-associated Fibroblasts and Suppresses Metastases in Orthotopic Triple Negative Breast Cancer Models

Mami Murakami, Mark J. Ernsting, Elijus Undzys, and Shyh-Dar Li. Drug Delivery and Formulation Group, Medicinal Chemistry Platform, Ontario Institute for Cancer Research, Toronto, Ontario, Canada

Purpose: Triple-negative breast cancer (TNBC) has a high distant recurrence rate after neoadjuvant chemotherapy, and there is currently no effective standard-of-care. Cancer-associated fibroblasts (CAF) are prominent mediators in breast tumor microenvironments, promoting tumor progression and metastasis. We have developed a docetaxel-loaded nanoparticle (Cellax, 120 nm), which selectively accumulates in the tumor with limited uptake by most normal tissues, and is efficiently internalized by cells in the tumor for sustained drug release for >10 days.[(1-3)] In this study, we compare the efficacy of Taxotere®, Abraxane® and Cellax in two orthotopic TNBC models.
Methods: Cellax were prepared as previously reported.[(1)] Carboxymethylcellulose (CMC) was acetylated and 37 wt% docetaxel and 5 wt% poly(ethylene glycol) (PEG) were coupled (Figure 1). Cellax particles were prepared by a nano-precipitation method, followed by dialysis and sterile filtration. Mice were inoculated with 4T1 or MDA-MB-231 cells in the mammary fat pad, and treated with Cellax, Taxotere® or Abraxane® in a neoadjuvant protocol. Metastatic incidence was monitored. In a separate study, stroma content, perfusion and interstitial fluid pressure (IFP) of the primary tumors were analyzed.

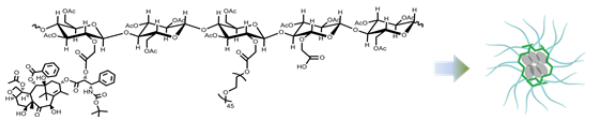


Figure Schematic representation of Cellax

Results: Cellax depleted stroma, reduced IFP and increased perfusion of the primary tumor, while Taxotere® and Abraxane® showed no effect. Treatment with Cellax reduced the incidence of lung metastasis to 40% without other visceral recurrence, while control, Taxotere® and Abraxane® treated animals displayed >85% incidence in lung metastasis with widespread metastases to bone, liver and kidney. The mice treated with Cellax exhibited no drug induced death, while 20-40% toxic death was observed with Taxotere® and Abraxane® therapy.

Conclusion: Our data suggest that Cellax treatment depleted CAF in the primary tumor and reduced metastases, demonstrating significant potential in improving TNBC therapy.

References

1. Ernsting MJ et al. *Bioconjug Chem* 2011;22:2474-86.
2. Ernsting MJ et al. *Biomaterials* 2012;33:1445-54.
3. Ernsting MJ et al. *Biomaterials* 2012;33: 3931-3941.

51. Gamma Irradiation of Ciprofloxacin in Solid State and In Gel Formulations: Physico-Chemical Characterization

Ibrahim El-Bagory. Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Purpose: The present work was based on studying the effect of gamma irradiation on the physico-chemical properties of Ciprofloxacin (CPX) in solid state as well as in different gel formulations.

Methods: The radiation doses were 0, 15, 25, 50 and 100 kGy, in both the solid and gel state. All the irradiated samples were subjected for physic-chemical testing such as DSC, X-ray, and SEM scanning.

Results: All irradiated samples in solid state showed chemical stability at the used doses. DSC thermogram showed no change in the melting point indicating that the CPX identity existed. These findings were also supported by the existence of principal absorption bands in the IR spectra. The decrease in the enthalpy by increasing the dose of irradiation attributed the change in crystalline ciprofloxacin to a more amorphous form. The X-ray diffraction patterns of irradiated solid showed a lesser degree of crystallinity as evidenced by fewer peaks of lower intensity compared with the non-irradiated sample. The characteristics of diffraction peaks relevant to crystalline CPX virtually disappeared by increasing the dose of radiation from I5 to 100 kGy. This was also clearly demonstrated by SEM photomicrography. In case of CPX gel using different concentration of Pluronic F-127, drug instability due to irradiation was less severe as concentration of pluronic was increased indicating more drug protection as the concentration of copolymer increased.

Conclusion: At pluronic concentration of 25% w/v, low irradiation doses namely 15 and 20 kGy did not harm the drug and its concentration in the gel was kept within 98 ± 1.59%. Sterility test on pluronic gel (25% w/v) revealed that irradiation dose at 20 kGy can give a definite sterile product.

52. Accelerated Blood Clearance of PLGA-PEG Nanoparticles Following Preceding Nanoparticle Injection: Influence of Polymer Dose and PEG

Roonak Saadati, Parvaneh Abbasian, Simin Dadashzadeh. School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Purpose: The accelerated blood clearance (ABC) phenomenon has been observed for PEGylated liposomes and some other nanostructures through the same mechanism. However, there are some recent reports which show the structure of PEGylated formulation can alter the ABC phenomenon. Therefore, the present study was performed to investigate whether the ABC effect is observed upon repeated injections of PLGA-PEG nanoparticles (NPs) as a very commonly used drug carrier. The effect of polymer dose and PEG and the production of anti-PEG IgM antibody as a potential reason for ABC phenomenon were also examined.

Methods: Etoposide loaded long-circulating PLGA-PEG NPs was developed by solvent evaporation method. Various pre-dose treatments, i.e. empty PLGA-PEG NPs at different polymer doses (over the range of 100 – 30000 µg), empty PLGA NPs and drug loaded PLGA-PEG NPs were injected as a first dose to male Wistar rats (250 ± 25 g). Seven days later one dose of drug loaded PLGA-PEG NPs was injected as the second dose (hereafter test dose). Control animals received etoposide containing PLGA-PEG nanoparticles without any first dose injection. At selected time intervals blood was sampled and plasma concentrations of etoposide were determined by a validated HPLC method. In

each examination the anti-PEG IgM antibody was also quantified.

Results: In the rats pre-administered with 100 μg of empty PLGA-PEG NPs the value of the etoposide AUC for the test dose strongly reduced ($p < 0.001$) and clearance was significantly enhanced ($p < 0.00001$) compared to the control (AUC: 3743.02 and 639.56 02 $\mu g*min*ml^{-1}$ and CL: 0.59 and 3.23 $ml*kg^{-1}*min^{-1}$ respectively). These changes were accompanied by a significant anti-PEG IgM production. These results clearly indicated the induction of ABC phenomenon by repeated injection of PLGA-PEG NPs. Increasing the polymer dose of the first injection from 100 to 20000 μg did not significantly affect the ABC, but a further increase of the polymer dose to 30000 μg noticeably affected pharmacokinetic parameters of etoposide. The non-significant change of etoposide clearance in pre-treated group by empty PLGA nanoparticles, compared to the control, confirmed the essential role of PEG in inducing the ABC phenomenon.

Conclusions: The results show that the ABC phenomenon is induced by PLGA-PEG NPs at certain intervals and anti-PEG antibody is the main possible reason for this phenomenon. However the polymer dose should be considered as an important factor for the design and therapeutic use of PLGA-PEG NPs for multiple drug therapy.

53. Influence of TPGS and PEG 400 on the Permeability of Etoposide across Everted Sacs of Rat Small Intestine

Simin Dadashzadeh[1], Hamid Parsa[1], Roonak Saadati[1], Parvaneh Abbasian[1], Saeed Azad[2]. [1]School of Pharmacy, Shahid Beheshti University of Medical Sciences, [2]Khatam Hospital, Tehran, Iran

Purpose: Etoposide, a widely used anticancer drug, exhibits low and variable oral bioavailability and the efflux transporter, P-glycoprotein (P-gp) has been considered as an important barrier against the intestinal absorption of this drug. Therefore, the present study was aimed to investigate the effect of D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and PEG 400 as p-gp inhibitors on the intestinal absorption of etoposide

Methods: Intestinal transport studies were examined by everted gut sac method. Everted sacs of rat small intestine were incubated in 25 ml of Krebs buffer solution which contained etoposide in the absence or presence of various concentrations of TPGS or PEG 400. The effect of verapamil as a known P-gp inhibitor on the absorption of drug was also studied. The solution was maintained at 37 ^oC with 95% O_2/5% Co_2. Samples from the solution inside the sacs were taken at predetermined times for 90 min. For exsorption studies etoposide was added into the sacs and samples were taken outside the sacs. The intestinal membrane damage was evaluated by measuring the release of LDH. Epithelial transport of a paracellular and a passive transcellular marker, lucifer yellow and imipramine respectively, in the absence and presence of excipients were also determined.

Results: The absorptive transport of etoposide was significantly enhanced ($p < 0.001$) in the presence of verapamil (100 μg/ml) and TPGS (over the concentration range of 0.002 – 0.1 mg/ml), however no significant change was observed by adding various concentration of PEG 400 (0.05, 0.1 and 0.5% w/v). No significant difference was found between permeability values in the absence and presence of maximum concentration of TPGS for transport markers, lucifer yellow and imipramine, indicating that enhancement in etoposide permeability in the presence of TPGS were not due to compromise in tight junctions or membrane integrity of epithelial cells.

Conclusions: The current data suggests that the use of TPGS as a safe excipient in etoposide formulations may enhance oral bioavailability of etoposide and result in a predictable oral absorption.

54. Sirolimus-loaded Stealth Colloidal Systems Attenuate Neointimal Hyperplasia after Balloon Injury: A Comparison of Liposomes and Phospholipid Based Micelles

Azadeh Haeri[1], Saeed Sadeghian[2], Shahram Rabbani[2], Maryam Sotoudeh Anvari[2], Simin Dadashzadeh[1, 3]. [1]Department of Pharmaceutics, School of Pharmacy, Shahid Beheshti University of Medical Sciences, [2]Department of Cardiology, Tehran Heart Center, Tehran University of Medical Sciences, [3]Pharmaceutical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Purpose: Restenosis after angioplasty remains a serious complication in clinical cardiology. Local delivery of anti-restenosis drugs encapsulated in biodegradable nanoparticulate systems with sustained release characteristics offers a potential

therapeutic approach to reduce restenosis following coronary angioplasty. This study aimed to investigate the stealth colloidal systems for local intra-arterial drug delivery.

Methods: Micelles from polyethylene glycol conjugated with phosphatidylethanolamine and PEGylated liposomes loaded with sirolimus (SIR), a lipophilic antiproliferative/immunosuppressive drug, were prepared and characterized with regard to their entrapment efficiency (EE), size distribution, zeta potential, drug release profile and stability. Antirestenotic effects of SIR loaded micelles (14 nm) and liposomes (90 nm) were evaluated and compared in the rat carotid injury model following local intravascular delivery.

Results: The results from the morphological studies including, hematoxylibasedn-eosin and Masson trichrome stain showed typical lesions that displayed significant neointimal proliferation in the control groups. The treatment of balloon injured rats with drug loaded micelles and nanoliposomes significantly influenced neointimal formation compared to control groups ($P < 0.05$). In addition, the luminal area of SIR nanocarriers treated groups was also significantly enlarged ($P < 0.05$). These results suggest that both colloidal nanocarriers could serve as effective drug delivery systems for the treatment of restenosis, however, the phospholipid based micelles provided better antirestenotic effects than the PEGylated liposomes; this is likely due to the distinctly smaller size of the phospholipid based micelles.

Conclusions: The proposed nanocarriers have dual action: as a drug releasing depot and biocompatible nanoparticulates which may reduce the problems caused by non-degradable as well as biodegradable polymers.

CSPT Posters - Day 1

Wednesday, June 13, 2012

CSPT Trainee Poster Presentation Competition Abstracts

55. Stability of Brominated Flame Retardants (PBDEs) Measured in Hair

Carnevale A, Aleksa K, Goodyer CG, Bagli DJ, Koren G (Hospital for Sick Children)

Background: Polybrominated diphenyl ethers (PBDEs) are chemicals added various consumer products as flame-retardants, and have been labeled as endocrine disruptors. They persist in the environment and studies suggest hair as a suitable matrix for examining human exposure. Since more than 80% of exposure is due to contaminated dust in our environment, we hypothesize that hair PBDE levels will be stable over time, as one's environment remains unchanged.
Objective: To measure the intra and inter-individual stability of PBDE levels detected in hair over a period of one year.
Methods: Hair from 22 females was collected at The Hospital for Sick Children as part of another study. To assess stability, hair samples were separated into four 3 cm segments, which represents a one-year period. Segments were analyzed for eight PBDE congeners, BDE-28,-47,-99,-100,-153,-154,-183 and -209 by GC-MS.
Results: The total ΣPBDEs (pg/mg) varied among individuals. Smaller amounts were observed in more recent segments; first (48.7±5.3), second (67.9±8.5), third (87.2±10.7), and fourth (138.3±15.6). BDE-47 (113±10.5) and BDE-99 (81.6±8.1) comprised of 59% of the total ΣPBDEs in hair.
Conclusion: As expected BDE-47 and 99 were the primary congeners. Results indicate that PBDEs are relatively stable over time. However, further data is needed to determine how PBDEs accumulate in hair over time as a result of multiple sources and pathways of exposure.

56. *Lactobacillus reuteri* DSM 17938 versus Placebo in the Treatment of Infantile Colic: A Randomized Double-blind Controlled Trial

Chau K, Jacobson S, Peer M, Taylor C, Greenberg S, Koren G (University of Toronto)

Background: Infantile colic is the most commonly reported medical problem in the first 3 months of life and although it is not a life-threatening condition, it causes appreciable distress for parents and pediatricians. The pathogenesis of colic remains unclear; however, multiple origins are suggested to be involved. Consequently, a variety of treatment options have been proposed, but there is currently no preferred approach to alleviate this condition.
Objectives: To investigate the effectiveness of the probiotic, *Lactobacillus reuteri* DSM 17938, in reducing infantile colic symptoms compared to a placebo treatment.
Methods: 100 infants diagnosed with either colic symptoms (>3 hrs of crying on >3 d/wk), fussy-gassy or gastroesophageal reflux (with or without esophagitis) will be enrolled in this randomized double-blind placebo controlled study. Colicky infants will be randomly assigned to receive *L. reuteri* or placebo at a standard dose of 10^8 colony-forming units 30 minutes following breast-feeding once daily for 21 days. The primary outcome is the reduction of mean crying time from baseline to the end of treatment with *L. reuteri* compared to a placebo treatment.
Discussion: A lack of characteristic manifestation of colic and the day-to-day variability of crying time, it is necessary to adopt a safe therapeutic approach to alleviate colic symptoms. As such, treatment with *L. reuteri* is believed to be a safe and effective treatment to treat infantile colic.

57. Functional Activity of CYP450 2J2, 3A5 and 2E1 in Human Heart Ventricles

Huguet J, Gaudette F, Michaud V, Turgeon J (Université de Montréal)

Background: Cardiac CYP450s functional activity may contribute significantly to the local metabolism of drugs. Using a cocktail of probe drugs we characterized CYP2J2, CYP3A5, CYP2E1 and CYP2B6 functional activities in human heart microsomes (HHM).
Methods: HHM were isolated by differential centrifugation from the human heart right ventricle. Ebastine (CYP2J2; 0 to 25 μM), chlorzoxazone (CZX) (CYP2E1; 0 to 630 μM), midazolam (MDZ) (CYP3A5: 0 to 5 μM) and bupropion (CYP2B6; 0 to 775 μM) constituted the substrate cocktail. Incubation mixture contained 100 mM Phosphate buffer, substrates and NADPH-generating system. Reaction was initiated by adding microsomal proteins. Samples were incubated at 37°C for 60 min. Metabolites were quantified by LC-MSMS.
Results: Investigation of in vitro Km and Vmax shows significant metabolism of CZX in 6-OH-CZX, of ebastine in OHebastine (Km; 0,52 μM, Vmax; 0,49 pmoles /min/ mg prot) and of MDZ in 1-OHMDZ (Km; 4,8 μM, Vmax; 0,01 pmoles /min/ mg prot). CZX did not achieve enzymatic saturation. CZX is also a substrate of CYP1B1 with similar affinity and mRNA relative expression shows higher level of CYP1B1 than 2E1. Activities measured in HHM for ebastine and MDZ correlated well with mRNA levels measured for CYP2J2 and CYP3A5, respectively. No activity was observed for CYP2B6.
Conclusion: HHM shows functional activities of CYP2J2, 3A5 and 2E1 which may contribute to the cardiac metabolism of drugs.

58. Oxycodone-induced Central Nervous System (CNS) Depression in Breastfed Neonates: Pharmacological Analysis

Lam J, Kelly L, Wurman C, Matok I, Ross CJ, Carleton BC, Hayden MR, Madadi P, Koren G (Hospital for Sick Children)

Introduction: Oxycodone is associated with an increased risk of CNS depression in both mothers and infants. The contribution of genetics in predicting toxicity in breastfeeding mother-infant pairs exposed to oxycodone is not known. The objective of this study was to assess the contribution of 18 polymorphisms in 4 genes involved in oxycodone metabolism and response in predicting both maternal and neonatal CNS depression.
Methods: A case-control study in 67 breastfeeding mother-infant pairs exposed to oxycodone was conducted. Cases were defined as parental reports of sleepiness in the infant temporally related to oxycodone exposure via breast milk. Maternal saliva samples were analyzed for 18 polymorphisms in 4 genes, *CYP2D6, CYP3A5, OPRM1, ABCB1* involved in oxycodone metabolism and response.
Results: Mothers of symptomatic infants were using oxycodone for a longer period of time during breastfeeding compared to asymptomatic infants ($p<0.0001$). None of the maternal genetic variants in the 4 genes were associated with oxycodone-induced depression in neonates. However, mothers carrying at least one copy of the *ABCB1 2677* T variant had an increased risk of experiencing sedation themselves [OR 2.35; 95% CI 1.06-5.28; p= 0.03].
Conclusions: Our study suggests that prolonged maternal use of oxycodone for greater than 4 days increases the risk of CNS depression in the breastfed newborn. Maternal *ABCB1 2677T* was identified to be a risk allele for experiencing maternal CNS depression.

59. Cocaethylene as a Biomarker in Human Hair of Concomitant Alcohol and Cocaine use in a High-risk Population

Natekar A, Matok I, Walasek P, Rao C, Clare-Fasullo G, Koren G (Hospital for Sick Children)

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 whether CE can be used as a biomarker in hair testing to indicate chronic excessive alcohol consumption.
Methods: We used liquid-liquid extraction and solid-phase microextraction 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: Of 588 individuals tested for cocaine and chronic alcohol abuse, 235 were positive for FAEE, indicating chronic excessive drinking. Of these, 99 individuals were also positive for cocaine use,

representing 42.1% of FAEE positive results. Positive hair cocaine predicted chronic alcohol consumption (OR 1.767 P<0.05). For logistic regression, FAEE and positive cocaine use had odds ratios of 2.44 and 15.56 respectively, for positive CE, indicating that positive FAEE and cocaine use predict positive CE results. Critically, positive CE results identified 90.2% of individuals who are considered chronic alcohol abusers (FAEE>=0.5 ng/mg).
Conclusions: In our cohort, CE had a 90.2% positive predictive value for chronic alcohol abuse and it can be used clinically for that end.

60. Characterization of Rytvela: an Allosteric Modulator of IL-1β-induced Inflammatory Processes

Noueihed B, Rivera C, Beauregard K, Quiniou C, Chemtob S (McGill University)

Background: Following an injury, Interleukin-1β (IL-1β) is secreted to regulate proinflammatory responses. It exerts its biological function by interacting with IL-1 receptor (IL-1R1) complex thus activating various downstream mediators. Of the current approved therapeutics, recombinant IL-1 receptor antagonist competes with IL-1β for receptor binding; however, numerous drawbacks limit its usage suggesting the need for small inhibitors offering preferable drug distribution. A short non-competitive IL-1R1 antagonist of the sequence rytvela, developed by our laboratory, was shown to be efficacious *in vivo.* Interestingly, it exhibits allosteric properties but its mechanism is unknown.
Objective: To study the effect of rytvela on IL-1β-regulatory actions.
Methods: The effect of rytvela on IL-1β-induced cytotoxicity in RGC-5 cells was measured using MTT assay. Furthermore, IL-1β-induced gene expression in rytvela-treated RGC-5 cells was analyzed using PCR. The suppression of IL-1β canonical NFκB and AP-1 pathways via rytvela was examined using NFκB/AP-1 reporter gene assay.
Results: rytvela restored viability in IL-1β-treated RGC-5 cells, supported by the reduction of caspase-3 expression. Moreover, it significantly decreased the gene expression of TNF-α, IL-6, and caspase-1; however, NFκB and AP-1 were unaffected.
Conclusion: rytvela can be an allosteric modulator of IL-1R1 function by selectively inhibiting downstream mediators without abolishing all signal transduction.

61. Comparative Performance of Sprague-Dawley Rat Hearts using DMSO and DMF as Cryoprotectants

Ozokwere J[1], Hazelhurst L[1], Olivier E[2], Letsoalo M[3] (Tshwane University of Technology)

Purpose: Heart transplantation is one of the most effective treatment options for congestive heart failure. Current organ storage methods can preserve the human heart for only about four to six hours. The organ donor pool could be dramatically increased if the preservation time could be lengthened and hearts stored for weeks or even month's prior to transplantation. This study describes the performance characteristics of explanted Sprague-Dawley rat hearts before and after cryopreservation using 10 % dimethylsulphoxide (DMSO) and 30 % dimethylformamide (DMF) in Tyrode solution.
Methods: A modified Morgan perfusion model was used for this study. Male Sprague- Dawley (ethical approval AREC/2009/09/002) hearts were harvested and arrested in a cold (< 10 °C) Tyrode solution (pH 7.4) for 5 minutes. The hearts were mounted on the aorta and vena cava to allow reperfusion in a doubled walled water jacket at 37 °C for baseline (Control) performance studies. The hearts (n=3) were cooled to 4 °C, -20 °C, -80 °C and -196 °C (liquid nitrogen), and stored for 6 hours. This study was extended to 48 hours and 7 days at -196 °C (n=6). Cardiac output (aortic and coronary) and an electrocardiogram were obtained during baseline studies, followed by cryopreservation and after thawing at times T0, 10, 20, 40, 60, 120 min, 6, 8, 12 and 24 hours. Reperfused hearts were monitored for as long as possible. Ethical approval (AREC/2009/09/002) for the use of laboratory animals was obtained from the Tshwane University of Technology, Ethics Committee and the Animal Ethics committee before experimental work commenced.
Results: The average heart rate of the Sprague-Dawley rats reduced from 396 beats / minutes to 184 beats / minutes after anaesthesia. The average survival time of the hearts under the experimental conditions were 7 hours 32 minutes with an average aortic output at 8 hours of 0.62 ml and 0.52 ml at 12 hours for DMF and 0.61 ml for 8 hours and 0.35 ml for DMSO at average survival time of 9 hours 44 minutes. A 100 % recovery after cryopreservation

with DMSO and DMF was achieved after storage for 6 hours, 48 hours and 7 days in liquid nitrogen. DMSO and DMF were equally effective cryoprotectants in this study. It was possible to preserve the hearts outside the body longer than 8 hrs as previously studied to 168 hour (7days) at –196 °C with 100 % recovery using both DMSO and DMF as cryoprotectant.

62. Quetiapine in Human Breast Milk – Population PK Analysis of Milk Levels and Simulated Infant Exposure

Tanoshima R, Yazdani-Brojeni P, Taguchi N, Garcia-Bournissen F, Moretti M, Verjee Z, Koren G, Ito S (Hospital for Sick Children)

Background: Quetiapine is one of the widely used atypical antipsychotic drugs in women of childbearing age. However, the information on quetiapine excretion into human milk is limited, and no population-based prediction exists.
Methods: A pharmacokinetic study was conducted in lactating women who were taking quetiapine. Multiple milk and single blood quetiapine concentrations were measured. Population PK analyses of milk quetiapine profiles were performed using non-linear mixed-effects method by directly modeling concentration profiles in milk without plasma level data. Using the final PK model, quetiapine milk concentrations at steady state of 1000 individuals were simulated. All modeling and simulations were conducted in NONMEM® VI and R.
Results: Nine subjects receiving fast-release quetiapine (mean dose: 40 mg/day) were analyzed at steady state. Mean milk/plasma ratio was 0.44 (range 0.098 -1.67). A two-compartment model with first order absorption was selected as the best model. Simulations based on the model parameters showed that 99% of the breastfed infants would ingest quetiapine at levels below 0.42% of the maternal weight-adjusted dose.
Conclusion: Infant exposure levels to quetiapine through breastfeeding are estimated to be very small. Based on the population simulation, we predict that the probability of significant exposure of the infant to quetiapine in milk is negligible.

63. Loss of Equilibrative Nucleoside Transporter 1 (ENT1) in Mice Leads to Progressive Ectopic Mineralization of Spinal Tissues Resembling Diffuse Idiopathic Skeletal Hyperostosis (DISH) in Humans

Warraich S, Bone DBJ, Quinonez D, Holdsworth DW, Drangova M, Dixon J, Séguin CA, Hammond JR (University of Western Ontario)

DISH is a non-inflammatory spondyloarthropathy, characterized by ectopic calcification of spinal tissues that occurs in 6-12% of North Americans, most over the age of 50. Its etiology is unknown and there are no specific treatments. *ENT1* mediates the Na^+-independent transport of hydrophilic nucleosides, such as adenosine, across plasma membranes. In mice lacking ENT1 (*ENT1*$^{-/-}$), we observed development of calcified lesions with remarkable resemblance to DISH in humans. MicroCT analyses revealed that *ENT1*$^{-/-}$ mice developed ectopic mineralization, starting in the cervical-thoracic spine and extending to the lumbar region with advancing age. Histological examination of decalcified samples revealed large, irregular accumulations of eosinophilic, amorphous material encapsulated by fibrocartilaginous cells, with no apparent inflammation. Plasma adenosine levels, determined by HPLC, were 2.8-fold greater in *ENT1*$^{-/-}$ compared to wild-type mice. In addition, quantitative RT-PCR analyses of spinal tissue from the cervical-thoracic region of 6-month-old mice revealed lower levels of adenosine A_1 receptor in *ENT1*$^{-/-}$ compared to wild-type mice. Lesions in the *ENT1*$^{-/-}$ mouse resemble DISH in humans and point to a role for purine metabolism in the regulation of biomineralization. *ENT1*$^{-/-}$ mice may provide a useful model to investigate mechanisms and to evaluate therapeutics for preventing pathological calcification in DISH and related disorders.

CIHR-DSEN Trainee Poster Presentation Award Abstracts

64. Human Hair Cortisol Analysis using an Elisa: A Comparison of the Different Reported Methods

Albar W[1], Russell E[1], Koren G[1,2,3,4], Rieder M[2,5], Van Uum S[2] (University of Western Ontario, Hospital for Sick Children, Children's Health Research Institute)

Background: Recently, hair cortisol analysis has been a topic of global interest among researchers. Therefore, the need for critical examination of the analytical methods has to be done in order to standardize the method and allow uniform interpretation.
Objective: To assess the similarities and differences among methods published to date.
Methods: This study compares among four published laboratories procedures: Drs.Van Rossum, Kirschbaum, Laudenslager, and Dr.Koren - Van Uum. We examined several common dimensions in their procedures.
Results: A major difference was the ELISA kit used. Alpco diagnostics (Salem, NH, USA) is used by Koren's group, Van Rossum uses DRG International, (USA) or DRG Instruments GmbH, (Marburg, Germany), Kirschbaum uses IBL (Hamburg-Germany), and Laudenslager uses Salimetrics, (LC). Koren and Van Rossum appear to have nearly the same mass of hair (10-15mg), do not wash the hair samples, have the same pulverization method which is mincing with surgical scissors, and the same amounts of the extraction and reconstituting solvents. In contrast, the other two groups use 50 mg of powdered hair and wash hair samples 2-3 times/3 minutes each with 2.5 ml isopropanol. Considerable other similarities were found.
Conclusions: Consensus toward developing one method that is comprehensive, convenient, and appropriate should be aimed.

65. Long-term Neurodevelopment of Children Exposed to Above Manufacturer Recommended Doses of Diclectin in Utero

Carey N, Koren G, Nulman I (The Hospital for Sick Children)

Background: Nausea and vomiting of pregnancy affects up to 90% of pregnancies. Doxylamine/pyridoxine is the only anti-emetic approved in Canada for NVP. The maximum dose is 4 tablets/day, at which lack of fetal toxicity, including longterm neurodevelopment has been established. However, some women receive higher doses, to 12 tablets/day. Although this dose has not been associated with major malformations, possible neurodevelopmental effects have not been investigated.
Methods: This is a prospective observational study. Four groups of mother-child pairs have been recruited: (1) NVP and>4 tablets (n=42), (2) NVP and <4 tablets (n=84), (3) NVP and no treatment (n=63) and (4) no NVP (n=49). At 6-9 months after birth, women were contacted for follow up. At ages 3-7, children received a full psychological assessment.
Results: All groups scored in the normal range for IQ and cognition tests. The NVP-exposed group scored significantly higher on the McCarthy numerical memory forward test (P<0.001), the NEPSY comprehension of instruction test (P=0.030), and the NEPSY visuomotor precision scale (P=0.010). Neither worst PUQE score or average dose were predictors of IQ.
Conclusion: Above manufacturer recommended doses of doxylamine/pyridoxine do not appear to harm neurodevelopment and should be considered safe for the treatment of NVP. NVP itself confers superior achievements in some tests among offspring of women with this condition.

66. Safety of Levetiracetam in Pregnancy

Chaudhry SA, Jong GW, Koren G (Hospital for Sick Children)

Background: Use of medications during pregnancy is often a challenge resulting in a lower compliance rate. For the medications where evidence is available and confirm the safety, women are more comfortable in continuing their use during pregnancy. There have been concerns regarding levetiracetam use during

pregnancy and adverse pregnancy outcome.
Objective: To systematically review the available published evidence on the safety of Levetiracetam use during pregnancy with focus on birth defects.
Method: Pubmed, Embase, and Cochrane library data base were searched for studies, including pregnancy registries, observational studies, and abstracts regarding the use of levetiracetam during pregnancy.
Results: The study population included pregnant women exposed to levetiracetam as a monotherapy or polytheray in the first trimester. Ten studies met the inclusion criteria, 5 were observational studies in the form of pregnancy registries and 5 were published in abstract form. There were no well controlled studies on the use of Levetiracetam in pregnancy as ethically hard to do. The available published data including from North American anti epileptic drug pregnancy registry, UK epilepsy and pregnancy registry, EURAP, the Dutch European registry of antiepileptic drugs, and Australian pregnancy registry showed 897 pregnancies exposed to levetiracetam. There were 20 major congenital malformation reported in these studies giving an overall risk of 2.2% (20/897). Although the exposure to monotherapy was associated with less risk 1.3% (8/604) as compare to poly therapy 4 % (12/296). Two studies have shown the development and language skills of exposed children at 3 to 24 months and 3 to 4 years were same like normal control.
Conclusion: The current evidence from all the registries suggests the overall risk of malformation after first trimester exposure are well within the baseline risk of 1-3 %. Long term developmental effects don't seem to be effected although the numbers are small.

67. Malaria Infection Alters Drug-disposition Mechanisms in Pregnancy

Cressman AM[1, 2], Silver KL[3], Kain KC[3], Piquette-Miller M[1, 2] (University of Toronto)

Background: Placental malaria has the potential to impact over 20 million pregnancies per year. Despite its high prevalence, little is known regarding the impact of malaria infection on drug-disposition mechanisms in pregnancy. As such, our objective was to characterize expression of key drug-disposition mechanisms in maternal, placental, and fetal tissues in an animal model of malaria.
Methods: 10^6 *P. berghei* ANKA-infected RBCs were injected i.v. into pregnant Balb/c mice at gestational day (GD) 13. Mice were monitored from GD13-19, sacrificed, checked for fetal viability, and maternal, placental, and fetal tissues were extracted and snap frozen from viable fetuses. RNA was isolated using TriZol reagent, assessed for purity using a NanoDrop, DNase I treated, and reverse transcribed to cDNA. Quantitative RT-PCR was used to assess changes in expression of drug transporters and drug-metabolizing enzymes from control and malaria-infected animals on GD19.
Results: Changes in expression of numerous drug-disposition mechanisms was observed in maternal liver, placenta, and fetal liver. Expression of placental transporters (Abcb1a, Abcb1b, Abcc1, Abcc2, Abcc3, Abcg2) was significantly down-regulated ($p < 0.05$). Expression of transporters in maternal liver illustrated significant and differential changes in the canalicular and basolateral hepatocyte domains. Expression of transporters in fetal liver revealed significant changes ($p < 0.05$), mirroring those observed in maternal liver. Both maternal and fetal Cyp3a11 were significantly down-regulated ($p < 0.01$).
Conclusions: Malaria-induced alterations in drug transporters and drug-metabolizing enzymes may significantly alter the pharmacokinetics of clinically-important therapeutics and other xenobiotics in pregnancy. Further studies are required to quantify the impact of these changes on maternofetal drug disposition.

68. Clinical and Transporter Pharmacogenetic Determinants of Plasma Atorvastatin and Rosuvastatin Concentrations in Patients

DeGorter MK, Tirona RG, Schwarz UI, Choi Y, Teft WA, Myers K, Suskin N, Zou G, Dresser GK, Hegele RA, Kim RB (University of Western Ontario)

Background: A significant barrier to statin therapy is muscle toxicity associated with elevated systemic exposure. Despite numerous clinical trials to assess statin safety and effectiveness, there is little data describing plasma levels in patients, or contribution of clinical and pharmacogenetic variables to the exposure required for optimal therapy.
Objective: To assess interindividual variability and determinants of statin levels in patients.
Methods: We measured atorvastatin and

rosuvastatin in 299 patients by LCMS, and evaluated the contribution of variants in transporter genes *SLCO1B1*, *SLCO1B3*, *SLCO2B1*, *ABCG2*, *ABCC2* and *ABCB1* by multiple linear regression.
R**esults:** There was 45-fold variation in statin concentrations among patients on the same dose. After adjusting for gender, age, BMI, ethnicity, dose and time from last dose, *SLCO1B1* c521T>C ($p<0.001$) and *ABCG2* c421C>A ($p<0.01$) were important to rosuvastatin concentration whereas *SLCO1B1* c388A>G ($p<0.01$) and c521T>C ($p<0.05$) were significant for atorvastatin. The p-hydroxyatorvastatin to atorvastatin ratio was significantly correlated with *SLCO1B1* c521T>C ($p<0.001$) and prescription of fibrates ($p<0.05$), known *SLCO* transport inhibitors. This finding was confirmed in *Slco1b2*$^{-/-}$ mice. **Conclusions:** There is significant variability in statin exposure associated with uptake and efflux transporter polymorphisms. There appears to be heterogeneity of the genotype-drug interaction across members of the statin class.

69. Fetal Safety of Cetirizine; A Prospective Cohort Study and MetaAanalysis

Etwel F, Djokanovic N, Moretti M, Boskovic R, Martinovic J, Koren G (University of Western Ontario)

Background: Cetirizine, a second-generation antihistamine, is a major active metabolite of hydroxyzine and is broadly used in the treatment of allergies, hay fever, angioedema, and urticaria, but the data on fetal safety are incomplete.
Methods: Pregnant women who were counseled by the Motherisk Program about cetirizine exposure in the first trimester were enrolled in a controlled, prospective cohort study and compared to pregnant women counseled about non-teratogenic exposures. A systematic review was conducted to identify and synthesize all cohort studies that examined pregnancy outcome of women exposed to hydroxyzine or cetirizine while pregnant. All the studies including the current study were combined in one meta-analysis using a random effects model.
Results: There were no significant differences in the rates of major malformations found between the cetirizine exposed and comparison group in both the cohort study (P=1.00) and the meta-analysis study (OR= 1.31, 95% CI: 0.99-1.73). In the meta-analysis there were also no differences in the rates of other pregnancy outcomes.
Conclusion: Hydroxyzine and cetirizine are not associated with an increased risk of major malformations or other adverse fetal outcomes.

70. CYP2C19, PON1, and the Role of PPIs in Clopidogrel Bioactivation and *in vivo* Antiplatelet Response

Gong IY, Crown N, Suen CM, Schwarz UI, Dresser GK, Knauer MJ, Sugiyama D, DeGorter MK, Woolsey S, Tirona RG, Kim RB (University of Western Ontario)

Background: Clopidogrel bioactivation and response has been associated with cytochrome P450 2C19 (CYP2C19). However, a recent study identified paraoxonase-1 (PON1) as the main driver of its bioactivation and efficacy. This study aimed to elucidate the contribution of PON1 and CYP2C19 to clopidogrel metabolism and response. Additionally, the interaction potential between clopidogrel and proton pump inhibitors (PPIs) was assessed.
Method: The influence of CYP2C19, PON1 and PPI coadministration, on clopidogrel active metabolite (H4) AUC and antiplatelet response was assessed in healthy subjects (n=21). The in vitro metabolic profiling of clopidogrel metabolism was conducted in microsomes.
Result: There was a remarkable correlation between H4 AUC and antiplatelet response (r2=0.78). Furthermore, CYP2C19 but not PON1 genotype was predictive of H4 AUC and response. There was no correlation between paraoxonase activity and H4 AUC. Coadministration of PPIs did not significantly alter H4 AUC or response. Metabolic profiling of clopidogrel in vitro confirmed the role of CYP2C19 in bioactivating clopidogrel to H4. Conversely, PON1 cannot generate H4, but mediates the formation of another thiol metabolite, Endo. Importantly, Endo plasma levels are nearly 20-fold lower than H4 and was not associated with response.
Conclusion: PON1 does not mediate clopidogrel active metabolite formation or antiplatelet action, while CYP2C19 remains a predictor of clopidogrel pharmacokinetics and response.

71. Hair Cortisol Concentrations in Patients with Obstructive Sleep Apnea

Russell EW, Koren G, Rieder MJ, Van Uum S (University of Western Ontario)

Background: Obstructive sleep apnea (OSA) is a common sleep disorder with serious cardiovascular and metabolic co-morbidities that may be mediated by increased cortisol secretion. Recent studies have focused on the ability of continuous positive airway pressure (CPAP) to reduce cortisol secretion in OSA patients, but the results have been mixed and only point measures of cortisol measurement have been used. Hair cortisol analysis presents a means of non-invasively and retrospectively examining cortisol production in these patients.
Hypothesis: Hair cortisol concentrations are increased in OSA patients, and may be decreased with successful intervention with CPAP.
Methods: Patients were recruited after undergoing a polysomnogram. Physical exam information and medical history were recorded. Polysomnogram data including the apnea-hypopnea index (AHI), total hypoxemic time, and arousals per hour were recorded before and after CPAP. Additionally, a hair sample and Perceived Stress Scale (PSS) were collected before and after CPAP. Hair cortisol concentrations were determined using our modified salivary cortisol ELISA protocol.
Results: Ninety-two patients were enrolled in the study, of which 31 returned after 3 months of CPAP therapy. A trend towards increased hair cortisol concentrations was noted in moderate and severe OSA patients ($P=0.056$). Hair cortisol concentrations were weakly negatively associated with total hypoxemic time ($r2=0.06$, $P<0.05$). Hair cortisol concentrations were not significantly changed after placement on CPAP, but perceived stress was significantly reduced ($P<0.001$).
Discussion: Cortisol secretion may be up-regulated in severe cases of OSA. The psychological stress of OSA may be reduced with CPAP, however physiological stress may remain.

CSPT Poster Presentation Abstracts

72. Development of Biomarkers for Pharmacogenetic Testing

Aminkeng F, Ross CJD, Visscher H, Rassekh RS, Pussegoda K, Amstutz U, Carleton BC, Hayden MR (University of Western Ontario)

Rationale*:* Cure rates for many diseases have improved significantly. Despite this, adverse drug reactions (ADRs) are a significant cause of morbidity and mortality. Genetic factors play a key role in the incidence and severity of ADRs. Pharmacogenomics aims to identify these factors.
Active ADR Surveillance: The Canadian Pharmacogenomic Network for Drug Safety (CPNDS) is a Canada-wide network using active surveillance to identify patients with severe ADRs with the goal of performing pharmacogenomic studies. The CPNDS consortium includes 20 sites across Canada. **Pharmacogenomic Predictions of ADRs*:*** Studies are designed, biomarkers panel is developed, patients are genotyped, raw data is processed and quality controls and association studies are performed. CPNDS has identified markers for anthracycline-induced cardiotoxicity (ACT), cisplatin-induced otoxicity (CIO), codeine-induced infant/child mortality, vincristine-induced peripheral neuropathy and carbamazepine-induced skin rash. GWAS and sequencing are ongoing to further validate these markers and identify new ones.
Functional Validation and Pharmacokinetic Evaluation: CPNDS has begun functional validation (in vitro and in vivo) and pharmacokinetic evaluation for identified genetic markers for CIO and ACT. Preliminary results show that the overexpression of SLC28A3 in human cardiomyocytes protects against ACT.
Discussion/Conclusion: Identifying/validating genetic markers for ADRs are imperative for improving drug safety.

73. Exposure to Statins During Pregnancy: Outcomes Over a 13 year Period

Davidovits J, Taguchi N, Rubin ET, Hosokawa A, Choi J, Yating Ying A, Moretti M, Laskin CA, Ito S, Koren G (Hospital for Sick Children)

Background: Statins decrease cholesterol synthesis by competitively inhibiting the rate limiting step in the cholesterol synthesis pathway. Currently, statins are contraindicated during pregnancy because postponement of hypercholesterolemia therapy during pregnancy is not believed to have long term negative health effects and early case reports of exposure described major fetal malformations. However, controlled cohort studies and further case reports have not validated their FDA category X pregnancy classification.
Objective: To evaluate the outcome of 13 years of pregnant Motherisk callers exposed to statins.
Method: A cohort study of pregnant women exposed to statins from 1998-2010. Pregnancy outcomes and birth defect information were gathered by phone interviews using a standard questionnaire. The primary outcome was the report of major birth defects.
Results: From 1998-2010, 112 exposed pregnant Motherisk callers inquired about statins. Statin use ranged from 4-19 weeks gestation. The exposure distribution was: 66 atorvastatin, 13 simvastatin, 25 rosuvastatin and 8 pravastatin. Outcomes collected to date are: 49 live births, 18 miscarriages, 3 abortions, and 1 neonatal death. No major malformations were reported, but 2 minor congenital anomalies were described: 1 soft tissue lump (surgically removed) and 1 case of ankyloglossia.
Conclusion: Statins do not appear to be major teratogens. However, further follow up is necessary as our sample size is small.

74. Riboflavin Treatment Reduces the Levels of BCRP-transported Drug Cimetidine into the Milk

Dedina L[1,4], Fujii H[2], Wu A[1,4], Harper P[3], Ito S[2,4]. (Hospital for Sick Children, University of Toronto)

Background: Mother's milk provides a multitude of benefits to the offspring. However, drugs and toxins that are transferred into breast milk may pose a risk to the nursing infant. The Breast Cancer Resistance Protein (BCRP) is known to actively transport drugs (e.g. cimetidine) and toxins (e.g. PhIP) into breast milk. BCRP also transports nutrients such as riboflavin into breast milk, and together with recently identified riboflavin transporters (RFTs), may provide a mechanism for riboflavin secretion into breast milk. It is currently unknown if RFTs are expressed in the mammary gland. Our objective was to characterize BCRP and RFTs expression in the mammary gland of FVB/N mice, and to investigate a potential strategy to decrease BCRP-transported xenobiotics excretion into the milk using a high-dose riboflavin intervention.
Methods: The expression of mRNA was analyzed using real-time quantitative PCR. Milk and plasma levels of riboflavin were quantified by high performance liquid chromatography. Levels of radioactive 3H-cimetidine in plasma and milk samples were determined by liquid scintillation counting.
Results: RFTs and BCRP expression was upregulated in the mammary gland of lactating mice. An intravenous injection of 5 μg/g body weight of riboflavin enhanced the levels of riboflavin in milk and plasma by 3.1- and 8.9–fold, respectively. Significant reduction in the levels of BCRP-transported 3H-cimetidine in milk was observed in the high-dose riboflavin treatment group.
Conclusion: RFTs upregulation in the lactating mammary gland suggests a potential role for these proteins in mammary riboflavin transport. Using riboflavin to exploit the function of mammary BCRP, significant reduction in milk levels of cimetidine was observed.

75. Hepatoprotective Activity of Berberis Aristata Root Extract against CCl4 induced Acute Hepatotoxicity in Rats

Dehar N[1], Walia R[1], Verma RB[1], Pandey P[2] (M.M. Institute of Medical Sciences and Research, Haryana, INDIA)

Background: CCL_4 is commonly used hepatotoxin in the experimental studies of liver diseases. Liver damage induced by carbontetrachloride (CCL_4) involves biotransformation of free radicals derivatives, increased lipid peroxidation and excessive cell death. *Berberis aristata* root extract, "Berberine chloride" is known to possess multiple pharmacological activities including anti-microbial, antiviral, anti-inflammatory, cholesterol lowering, anti cancer and anti-oxidant effects. The present

study was conducted to evaluate the hepatoprotective activity of berberine in CCL_4 induced hepatotoxicity in rats.

Material & Methods: The experimental protocol was approved be the IAEC. Adult wistar rats aged 7-9 weeks were injected intraperitoneally with 50% CCl_4 as 1:1 mixture in liquid paraffin. Berberine was administered i/p before or after CCl_4 treatment in various groups. Twenty-four hours after CCl_4 injection, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP) activities, total serum bilirubun levels and liver weight were measured. Histological changes of liver were examined with microscopy.

Results: The serum levels of AST, ALT, ALP and T. bilirubin were significantly increased ($p<0.01$) in CCl_4 treated group 2 rats. Group 3-5 rats treated with CCl_4 followed by Berberine chloride at doses of 5, 10 and 20 mg/kg, i/p respectively showed significant decrease ($p<0.05$) in AST, ALT, ALP and T. bilirubin levels when compared to group 2 in a dose-dependent manner. The percentage reduction of biochemical parameters after treatment with Berberine at 5, 10, 20 mg/kg in group 3-5 showed significant results ($p<0.05$) in AST (35%, 86%, 93%), ALT (50%, 87%, 91%), ALP (32%, 72%, 83), T. bilirubin (90%, 53%, 72%) and liver weight reduced to 6%, 12%, 20%. Histological examination showed lowered liver damage in berberine-treated groups.

Conclusion: The present study demonstrates that berberine possesses hepatoprotective effects against CCl_4-induced hepatotoxicity and that the effects are both preventive and curative. Berberine should have potential for developing a new drug to treat liver toxicity.

76. Metabolic Ratio of Opioids in Hair: A Novel Method to Study Population Genetic Polymorphisms

Delano K, Walasek P, Aleksa K, Koren G (Hospital for Sick Children)

Background: Codeine, still widely prescribed for its analgesic effects, is subject to CYP2D6 polymorphisms affecting its metabolism. Metabolic ratio (MR) of morphine to codeine represents the extent of codeine metabolism to its active metabolite. These compounds can be found in hair, which has not been used as a matrix to study MR. Studying MR in hair can provide a simple method to evaluate population variability obviating the need for blood.

Methods: Hair samples were collected from the Motherisk Laboratory for testing as per request by social workers and other agencies. From July 2010 to December 2011, 1966 samples were tested for codeine and morphine through GC-MS analysis. All codeine positive samples and respective morphine results were used to calculate MR.

Results: Of the 239 codeine positive samples, 192 had an MR of 0, indicating no morphine detection. Nineteen samples had an MR <1 while 15 samples with an MR between 1 and 2 were found. In addition, 5 samples with an MR between 2 and 3 were detected and 8 samples were found to have an MR >3. Contrasting these data to blood metabolic ratios will allow for validation of this method.

Conclusion: From these results, wide differential incorporation of codeine and morphine into human hair has been found. With better understanding of codeine and morphine incorporation into human hair, polymorphisms can be studied in hair using MR. This is the first study to assess the use of human hair as a matrix to study polymorphisms.

77. Acute Hepatotoxic and Inflammatory Responses to Liver Carcinogens in the Developing Mouse

Hanna D, Grant DM (University of Toronto)

4-Aminobiphenyl (ABP) is an aromatic amine procarcinogen found industrially and in cigarette smoke. ABP bioactivation may involve initial *N*-oxidation by CYP1A2 followed by *N*-acetyltransferase- (NAT) mediated conjugation to an intermediate that decomposes to a DNA-binding nitrenium ion. Neonatal exposure to ABP causes liver tumours in male but not female C57BL/6 mice, but NAT-deficient *Nat1/2(-/-)* mice are protected. However, biomarkers of DNA damage do not differ between sexes or strains. Diethylnitrosamine (DEN) shows the same sex difference as ABP. Adult mice exposed to DEN show an estrogen-dependent sex difference in acute hepatotoxic and inflammatory responses, suggesting a role for these processes in its carcinogenesis. We hypothesize that neonatal exposure to either ABP or DEN will cause sex and strain differences in acute hepatotoxicity. Mice were exposed to ABP and DEN using a tumor-generating dose. Mice were also exposed to DEN (25 mg/kg) using an established tumor-inducing protocol.

Markers of liver damage (alanine aminotransferase (ALT) activity) and inflammation (interleukin-6 (IL-6) expression) were assessed. ABP or DEN treated mice had no treatment, sex or strain differences in ALT activity or IL-6 expression. Mice treated with 25 mg/kg DEN had increased IL-6 expression but no sex difference. Our results suggest that low doses of ABP and DEN are poor hepatotoxins, and that increased IL-6 in DEN treated mice may be due to dose and chemical specific factors.

78. Chemokine-like Receptor 1 Regulates Myogenic Differentiation *in vitro* and *in vivo*

Issa M, Shanmugam M, Ernst MC, Parlee SD, Zabel BA, Butcher EC, Sinal CJ, Goralski KB (Dalhousie University)

Background: The chemokine-like receptor-1 (CMKLR1) is a G-protein coupled receptor that is activated by the adipokine chemerin. Previous studies have shown that CMKLR1 is highly expressed in white adipose tissue and liver, and plays an important role in the regulation of adipogenesis and osteoblastogenesis. Based on the established function of CMKLR1 in cell differentiation we invetigated the hypothesis that CMKLR1 regulates the differentiation of myoblasts into myotubes both *in vitro* and *in vivo*.
Methods and Results: In C2C12 mouse myoblasts, *CMKLR1* expression increased 3-fold with differentiation into multi-nucleated myotubes. Abolishing CMKLR1 expression, using an adenoviral-delivered shRNA, impaired the differentiation of C2C12 myoblasts into mature myotubes and reduced the expression of myogenic regulatory factors *myogenin* and *MyoD* as measured by quantitative real time PCR Embryonic (E12.5) CMKLR1$^{-/-}$ mice displayed significantly lower wet weights and a considerably diminished myotomal component of somites as revealed by immunolocalization of myosin heavy chain protein. These changes were associated with increased Myf5 expression in both C2C12 myoblasts and in mouse E12.5 embryos. Adult male CMKLR1 knockout mice exhibited significantly reduced bone-free lean mass and weighed less than the CMKLR1-expressing mice.
Conclusion: We conclude that CMKLR1 plays an essential role in myogenic differentiation of C2C12 cells *in vitro* and the CMKLR1 null mice exhibit a subtle skeletal muscle deficit beginning from embryonic life which persists during postnatal life.

79. Neonatal Adverse Effects Following Exposure to Benzodiazepines during Breastfeeding

Kelly LE, Poon S, Madadi P, Koren G (University of Western Ontario)

Background: Breast milk provides newborns with the ideal form of nutrition. The choice to initiate breastfeeding while taking medications chronically is a decision faced by many new mothers. Benzodiazepines are commonly prescribed anxiolytic agents and they have been detected in breast milk. Some studies suggest possible harmful effects in the suckling infant.
Objective: of this study was to assess the central nervous system depression and other adverse effects in infants exposed to benzodiazepines through breast milk.
Methods: Mothers who contacted the Motherisk program regarding the safety of benzodiazepines were invited to participate in a follow-up program regarding the effects of benzodiazepines on their infants during lactation.
Results: A total of 124 consenting women participated. Adverse outcomes, specifically sedation was identified in only 1.6% (2/124) of babies and was not associated with benzodiazepine dose, number of hours breastfed, or any demographic trait. Mothers reporting adverse outcomes in themselves [26% (32/124)] were more likely to be taking concomitantly a greater number of CNS depressants.
Conclusion: This study supports the continued recommendation to initiate breastfeeding while taking benzodiazepines postpartum.

80. Techniques to Answering Epidemiological Related Health Questions about Pregnancy

Kennedy DA, Koren G (The Hospital for Sick Children)

Background: Policy makers, researchers, clinicians and the public look for answers to population health related questions, yet reading a report of one randomized clinical trial (RCT) would often not provide a comprehensive picture to base critical decisions. Often, only observational studies are available. In pregnancy, RCTs are very rarely

conducted.
Objectives: Describe several methods currently available to obtain answers to health related questions where RCTs do not exist or may provide insufficient information.
Methods: Experience drawn from our recently completed projects, supplemented by other reference materials will be used to describe techniques to answer population health related questions.
Results: Registries, administrative and population databases, such as the World Health Organization's (WHO) databases can be important sources of data. For example, the WHO's databases can be used to compare trends across countries over time; comparing outcomes where practices/environment may differ. Systematic review and meta-analysis techniques can be used to bring together outcomes from different studies; providing more robust answers. The strengths and weakness of the various sources and techniques are discussed along with key aspects to pay attention to when conducting the analysis. **Conclusions:** RCTs are not usually available to answer population related questions in pregnancy; however, alternative sources and techniques are available to answer these types of questions.

81. Genotype Specific Approaches to Preventing Drug-induced Liver Injury in Multiple Sclerosis: Work in Progress

Kowalec KA, Ross CJ, Smith A, Traboulsee A, Yoshida E, Marrie R, Hayden MR, Carleton BC, Helen T (University of British Columbia)

Multiple sclerosis (MS) is a neurodegenerative disease and the most common cause of neurological disability in young adults in the Western world. Interferon-beta (IFNβ) is widely prescribed for treating MS, yet its effectiveness is modest. A common adverse reaction associated with its use is the development of abnormal biochemical liver test results. Despite regular monitoring, cases of liver failure, necessitating a transplant are reported. We aim to identify genetic variants associated with IFNβ-induced liver injury, with the ultimate goal of predicting an individual's risk of developing this serious adverse reaction.
Hypothesis: There are genotypic differences between those who develop liver injury, compared to those who do not.
Protocol: Cases are defined as MS patients reaching the clinical threshold for drug-induced liver injury. Controls are matched to cases based on age, sex, area of residence and having taken IFNβ for >2 years with no evidence of drug-induced liver injury. Each patient's DNA will be genotyped to identify genetic variants associated with susceptibility to IFNβ-induced liver injury. Genotyping will be done using a panel of adverse drug reaction (ADR) - related genes consisting of HapMap tag SNPs and functional gene variants and CNV-specific assays for genes with known deletion and duplication variants. To date, 15 cases and 59 controls have been enrolled from BC, with recruitment beginning in Manitoba and London, Ontario by mid-2012.

82. Drug-drug Interactions between Rosuvastatin and β-blockers Through the OATP1A2 Transporter

Lu J, Leung Y, Gaudette F, Turgeon J (University of Montreal)

Background: OATP1A2 is a membrane transporter potentially involved in the absorption of various drugs, such as rosuvastatin and β-blockers. The concomitant use of more than one substrate of OATP1A2 can result in a drug-drug interaction and this could modify the pharmacokinetic profile of the medications. The goal of this study was to determine whether there is interaction in vitro between rosuvastatin and several β-blockers through OATP1A2.
Methods: A HEK293 cell line overexpressing OATP1A2 was used as model for the study. First, the cells were grown to confluence on 12-well plates. Then, they were co-incubated in the presence of rosuvastatin and increasing concentrations of the β-blockers: carvedilol, alprenolol, metoprolol, propranolol, timolol, acebutolol, celiprolol, nadolol, atenolol, and sotalol. The amount of rosuvastatin transported in the cells was measured by UV-HPLC.
Results: The β-blockers carvedilol, alprenolol, metoprolol, propranolol, and timolol were capable of inhibiting the uptake of rosuvastatin through OATP1A2 with IC_{50} of 7.7, 40.0, 51.0, 51.6, and 53.8μM, respectively.
Conclusions: This study shows that a drug-drug interaction exists in vitro between rosuvastatin and several β-blockers, where carvedilol is the most potent inhibitor. Such an interaction may potentially occur under regular administration of those drugs. Consequently, some β-blockers may modulate

absorption, distribution and metabolism of OATP1A2 substrates.

83. The Role of Monolysocardiolipin Acyl Transferase - 1 (MLCL AT-1) in Barth Syndrome

Mejia EM, Hatch GM, Taylor W, Vandel M (University of Manitoba)

Background: Barth Syndrome (BTHS) is a rare X-linked genetic disorder caused by mutations in the *TAZ* gene. The *TAZ* gene product, tafazzin, is responsible for remodeling Cardiolipin (CL) with the necessary acyl species. *TAZ* mutations can result in cardiomyopathy. Our laboratory has discovered a new enzyme involved in the remodeling of human CL, monolysocardiolipin acyltransferase-1 (MLCL AT-1). **Objective:** We will examine if MLCL AT-1 complements tafazzin in the remodeling of CL.
Methods: Epstein-Barr virus transformed lymphoblasts from normal or BTHS patients were transfected with either *TAZ* RNAi or *MLCL AT-1* RNAi. Other groups were transfected with both *TAZ* RNAi and *MLCL AT-1* RNAi while the remaining groups were transfected with *TAZ* RNAi or *MLCL AT-1* RNAi plus an *MLCL AT-1* gene containing plasmid. *TAZ* and *MLCL AT-1* gene expression were analyzed using RT PCR. CL mass, MLCL AT-1 enzyme activity and incorporation of [1-^{14}C] Linoleic acid into CL were also analysed.
Results: *MLCL AT-1* gene expression increased when *TAZ* was knocked down. Expression of *MLCL AT-1* restored CL levels, increased [1-^{14}C] Linoleic acid incorporation into CL, and raised MLCL AT-1 enzyme activity in normal and BTHS cells in which *TAZ* was knocked down. Knockdown of *MLCL AT-1* and *TAZ* in normal or BTHS cells did not reduce MLCL AT-1 activity greater than knockdown of *TAZ* alone.
Conclusion: *MLCL AT-1* expression in BTHS cells may serve as a potential therapeutic approach to treat BTHS.

84. Absolute Quantification of P-glycoprotein Drug Transport Activity for in vitro to in vivo Pharmacokinetic Prediction

Morgan AD (The University of Western Ontario)

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. P-gp transport of probe drug sitagliptin was examined in a model of cultured, polarized epithelial cells heterologously expressing varying amounts of transporter using adenoviral gene delivery. 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. 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.

85. Roles of Cysteine 378 and Cysteine 416 in Human Equilibrative Nucleoside Transporter 1 Function and Ligand Interactions

Park JS, Hammond JR (The University of Western Ontario)

Nucleosides and their analogues require specific transporters to enable their movement in and out of cells. Human equilibrative nucleoside transporter 1 (hENT1), is a bi-directional facilitative transporter with an 11 transmembrane topology. Previously, with heterologous expression in mammalian cells we showed hENT1 sensitivity to charged

methanethiosulfonate (MTS) modifications. Positively charged MTSET inhibited binding of the prototypical ENT1 inhibitor [^{3}H]NBMPR while negatively charged MTSES affected its binding exclusively in cell membranes. In this study, we use cysteine-directed mutagenesis to identify residues responsible for charged MTS sensitivity. Mutation of the predicted extracellular C378 abolished MTSET effects seen in wild-type while mutation of C414 located in intracellular loop 5 (IL5) resulted in an enhancement of these effects. A double mutation of both C378 and C414 removed the inhibition of MTSET to [^{3}H]NBMPR binding indicating a structural link between the two residues. Loss of the cytoplasmic C416 (IL5) lead to a complete loss in [^{3}H]2-chloroadenosine transport, a classical substrate for ENT1. Additionally, C416S mutant was insensitive to MTSES treatment in broken cells identifying it as the residue reacting with MTSES to decrease [^{3}H]NBMPR binding. Taken together, we have identified two areas of importance in hENT1, the extracellular C378 and cytoplasmic C416 as residues contributing to inhibitor binding and substrate translocation sites.

86. Pharmacogenetics and Pain Management: A Review

Shaw JLV, Kapur B (University of Toronto)

Objectives: To review the literature surrounding the role for pharmacogenetic (PG) testing in the medical management of patients with chronic pain.
Methods: A systematic literature review was performed.
Results: Current treatment strategies for chronic pain follow the WHO pain ladder and opioids remain the mainstay of medical treatment for chronic pain, both cancer and non-cancer. Several genes have been shown to be associated with altered PK and PD of certain pain management drugs. The majority of studies of PG testing in pain management patients focus on one gene and its affect on drug metabolism in isolation. There is a paucity of evidence that PG testing in chronic pain patients results in better analgesia with less adverse drug effects. There are data to suggest that monitoring of serum drug and/or metabolite levels may lead to better patient outcomes.
Conclusions: PG only partially explains the altered drug metabolism between individuals. Furthermore, the metabolism of most drugs involves more than one enzyme and can therefore be affected by multiple genes. The metabolism of pain medications can also be affected by many other factors, such as drug-drug interactions, dosing, disease co-morbidity, nutritional status, gender and age. Measurement of serum drug and metabolite concentrations likely provides more individualized information about the drug metabolism in a patient, since serum concentrations account for all of the various factors affecting drug metabolism.

87. Safety of Inhaled Corticosteroids during Pregnancy

Smy L, Koren G (University of Toronto/Hospital for Sick Children)

Background: Asthma is the most frequent chronic condition occurring during pregnancy but few prospective studies have been done to assess the safety of inhaled corticosteroids (ICSs) during this time. Pregnant women refrain from taking their ICS for fear of harming their baby, but poorly controlled asthma is also linked with adverse perinatal outcomes, such as prematurity or low birth weight. Oral corticosteroid therapy is associated with adverse drug reactions (ADRs) such as adrenal suppression, gastrointestinal complications, or decreased immunity. While inhaled corticosteroids are felt to be safer, some of the same ADRs as oral therapy have been found with their use.
Objectives: To assess maternal and fetal safety by employing active surveillance (AS), carried out by trained individuals, to identify maternal ADRs potentially linked with ICS use during pregnancy. Additionally, to collect detailed clinical and drug history, and prenatal outcome information from the pregnant women.
Methods: Using AS, a total of 150 asthmatic pregnant women on ICS therapy, and 75 negative controls (those not on ICS therapy), will be recruited through local asthma clinics and the Motherisk Program at SickKids Hospital. Assessment of ADRs will be by questionnaire. Where possible, chart reviews will be conducted, and hair samples collected to test cortisol levels as a sign of adrenal suppression.
Outcome: It is hoped evidence will show ADRs do not occur with ICS use during pregnancy.

88. Dehydroepiandrosterone Alters Tocopherol Levels and Expression of Tocopherol Transfer Protein

Takitani K, Miyazaki H, Tamai H (Osaka Medical College, Takatuki, Japan)

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. Vitamin E, which has a critical role as a lipid-soluble antioxidant and prevents lipid peroxidation in a variety of tissues in several pathological conditions.
Objectives: In this study, we examined vitamin E status in rats administered DHEA and investigated the expression of vitamin E related proteins including alpha-tocopherol protein (alpha-TTP), which binds selectively alpha-tocopherol, and regulates the distribution of tocopherol in the plasma and various peripheral tissues.
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: Plasma alpha-tocopherol level in DHEA administered rats are increased compared with controls. Hepatic alpha-TTP gene expression is significantly increased in DHEA administered rats. Expression of alpha-TTP may affect circulatory vitamin E status.
Conclusions: DHEA and DHEA-S are widespread as supplements for anti-aging. DEHA may have a synergetic effect as anti-oxidants with vitamin E.
.

Poster Presentations
Day 2
Thursday, June 14, 2012

CSPS Posters - Day 2

Thursday, June 14, 2012

Biomedical Sciences

89. Dehydroepiandrosterone Esters having an Imidazole Ring at C-17 as Antiandrogens

Mariana Garrido[a], Marisa Cabeza[b], Eugene Bratoeff[a], Department of Pharmacy, Faculty of Chemistry, National University of Mexico City[a], Mexico D.F., Mexico; Department of Biological Systems and Animal Production, Metropolitan University[b]-Xochimilco, Mexico D.F.,Mexico,

Purpose: In this study, we describe the synthesis of several new dehydroepiandrosterone derivatives having a imidazole ring at C-17, cyclic aliphatic ester at C-3 (cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl , cyclopheptyl) and a conjugated formyl group at C-17. In view of the fact that the testosterone in the body is converted to dihydrotestosterone which causes prostate cancer, benign prostatic hyperplasia and other androgen dependent diseases; it follows that the inhibition of androgen formation by antiandrogens (antagonists to the androgen receptor) or inhibition of 17α-hydroxylase a C_{17},C_{20}-lyase enzyme plays an important role in androgen hormone biosynthesis. A complete inhibition of this enzyme could block androgen formation and as a result of this, inhibitors of this enzyme could be used for the treatment of androgen dependent diseases.
Method: In this work, dehydroepiandrosterone was used as the starting material for the synthesis of 3β-acyloyloxy-16-formyl-17-(1H-1,3-imidazole-1-yl)androsta-5,16-diene derivatives. In the first step of this synthesis, the 3β-hydroxyl group of the steroid was treated with the corresponding cyclic aliphatic acid, followed by a Vilsmeyer-Haack formylation at C-16. Subsequently, the imidazole ring was introduced at C-17 position. This reaction sequence resulted in the formation of five new dehydroepiandrosterone derivatives. The biological activity of these compounds was determined in vitro (inhibition of CYP-17 enzyme) and cancer cell lines.
Results: The five new esters were obtained in high yields and identified by IR, NMR (1H, ^{13}C) and mass spectrometry. The biological evaluation of these compounds indicated that the steroidal derivative having the cyclopropyloyloxy ester side chain at C-3 showed the highest activity in all biological models tested as compared to the commercially available CYP-17 inhibitor ketoconazol. This compound exhibited also the highest prostate cancer cell proliferation inhibition.
Conclusion: In this study, we report the synthesis and pharmacological evaluation of several new dehydroepiandrosterone compounds. The steroidal derivative having the cyclopopyloyloxy side chain 3b-cyclopropyloyloxy-16-formyl-17-(1H-1,3-imidazole-1-yl)androsta-5,16-diene showed the highest activity in all biological models tested. The results of this pharmacological study indicated that this compound could be a suitable candidate in the future for the treatment of androgen dependent diseases.

90. *In vitro* and *in vivo* Studies of a New pH Sensitive Starch Based Nanoparticulate MRI Contrast Agent

Alireza Shalviri[1], Warren D. Foltz[2], Ping Cai[1], Gaurav Raval[1], Heiko Heerklotz[1], Andrew Mike Rauth[1], Xiao Yu Wu[1]. [1]Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON; [2]STTARR Innovation Centre, Department of Radiation Oncology, Princess Margaret Hospital, Toronto, ON, Canada,

Purpose*:* Current clinically used low-molecular weight MRI contrast agents such as Magnevist® and Omniscan® have exhibited several disadvantages such as relatively low T_1 relaxivity (r_1), non-specific distribution to the whole body, and fast renal clearance. The objective of this study was thus to develop a new macromolecular MRI contrast agent from biocompatible and biodegradable starch with improved contrast enhancement capability and favorable pharmacokinetic properties.
Methods*:* Poly(methacrylic acid)-grafted-starch-DTPA (PMAA-g-St-DTPA) nanoparticles was

synthesized using a one-pot synthesis process, developed in our laboratory, which enabled simultaneous grafting and nanoparticle formation in water. The new starch based nanoparticulate contrast agent consisted of a polysaccharide backbone covalently linked to multiple chelating groups (DTPA) for gadolinium binding. The nanoparticles were characterized by DLS, TEM, ITC, ICP and their T_1 relaxivity was measured in pH 5-7.4 at a magnetic field strength of 3.0 T. The toxicity of the Gd-nanoparticles was evaluated *in vitro* using rat hepatocytes. The biodistribution and tumor accumulation of the particles were examined *in vivo* in a Balb/c mouse model.

Results*:* The nanoparticles with 17.3% (w/w) Gd^{3+} loading had an average size of 194 ± 23 nm and zeta potential of -21.8 ± 2.2 mV. The particles were relatively monodispersed with PdI= 0.1 and had a spherical shape. The particles remained stable up to 7 days in a physiologically relevant medium (0.9% NaCl + 10% FBS) with no sign of particle aggregation. The T_1 relaxivity of Gd^{3+} loaded PMAA-g-St-DTPA nanoparticles was nearly two-fold that of Omniscan®. The nanoparticles showed pH dependent relaxivity. The Gd^{3+} loaded nanoparticles did not cause any toxicity in rat hepatocytes. The particles showed superior and prolonged whole body contrast enhancement *in vivo* at one-fourth the equivalent Gd^{3+} dose of Omniscan®.

Conclusion*:* Our results suggest that this new nanoparticulate contrast agent has the potential for diverse and extensive applications in MR imaging.

91. Analyzing the Potential Neuroprotective Effects of Antioxidants

Stephanie Hewitt, Catherine Orr, Lyudmila Chibrikova, Skye Fisher and John T. Weber. School of Pharmacy, Memorial University of Newfoundland

Purpose: The accumulation of reactive oxygen species in the human body can causes oxidative stress, which is believed to contribute to aging as well as cell damage and death in neuropathologies such as stroke and traumatic brain injury (Slemmer *et al*., 2008). Foods containing particularly high amounts of antioxidants (e.g. polyphenols in berries) offer a potential means of protection against traumatic insults and the normal aging process. In this project, extracts of native Newfoundland and Labrador bilberries and oxyresveratrol, a potent antioxidant derived from mulberry wood, were analyzed for neuroprotective ability *in vitro*.

Methods: Primary cell cultures were produced from the cortex of neonatal rats. Cell health was monitored in untreated cells and in the presence of bilberry extracts or oxyresveratrol, which were added to culture media for various lengths of time. An *in vitro* model of traumatic injury was used to produce linear mechanical strain (Ellis et al., 1995). Immunohistochemical stains that were used include DAPI, which labels the nuclei of all cells, as well as MAP-2 and NeuN, which label only neurons. Lactate was measured in media using a multi-assay analyzer.

Results: Trauma was induced in neurons and glial cells by stretch injury, which resulted in significant neuronal death. Cultures were treated with oxyresveratrol (25, 50 or 100 μM) or bilberry extracts at the time of injury. There was no significant effect on glia and neurons with the addition of bilberry extracts in the trauma model. However, oxyresveratrol demonstrated protection against stretch-induced cell damage. A high level of lactate was observed in media after traumatic injury, indicating that cells were unhealthy. When bilberry extracts were added to cultures 15 minutes before injury lactate levels dropped significantly. Cultures treated with 100 μM oxyresveratrol had a significantly lower amount of lactate release than controls 24 hrs after the injury.

Conclusion: Trauma produced significant cell damage and an increase in lactate release. Bilberry extracts displayed some protection as evidenced by a decrease in lactate levels. Interestingly, it was found that oxyresveratrol strongly protected neurons from stretch-induced trauma. Although further experimentation is necessary, these results suggest that antioxidant compounds derived from natural products may offer protection from insults such as traumatic brain injury or brain aging.

Acknowledgements: Funding from NSERC supports this research. Stephanie Hewitt is a recipient of a CSPS 2012 National Summer Student Research Program Award sponsored by GlaxoSmithKline Inc.

92. D-Galactosamine Increases Bone Morphogenetic Protein 4 Expression in Human Hepatocellular Carcinoma Cells

Xiaotong Wang, Weisi Kong, Yuewen Gong. Faculty of Pharmacy, University of Manitoba, Winnipeg, MB

Background: Bone morphogenetic protein 4 (BMP4) is a member of transforming growth factor beta (TGF-b) superfamily. Previous studies indicated the increase in BMP4 mRNA and protein levels in bile duct ligated liver. However the functions of BMP4 in the liver still remain unclear. The current study investigates expression of BMP4 by hepatotoxin in one human hepatocellular carcinoma cell line (Huh-7 cells).
Methods: Huh-7 cells were cultured in DMEM with 5% fetal bovine serum and treated with D-Galactosamine (D-Gal) in different times and doses. The expression of BMP4 was examined by both real time RT-PCR and western blot analysis. Cell proliferation was evaluated by cell doubling time. Cell morphology was taken by an inverted microscope with Nikon camera.
Results: D-Gal induced BMP4 mRNA expression in a time and dose dependent pattern. BMP4 mRNA level reached peak at 6 hours treatment of D-gal and gradually decreased to normal level at 48 hours. In addition, D-Gal inhibited Huh-7 cell proliferation (doubling time from 31.2 hours to 43.2 hours). At the concentrations used, D-Gal did not induce significant change of Huh-7 cell morphology.
Conclusion: D-Gal significantly inhibits Huh-7 cell proliferation and induces bone morphogenetic protein 4 expression at the same time. However it still remain to determine whether increased expression of BMP4 contributes to D-Gal inhibition of cell proliferation.

93. Metformin Protects Against DMBA-induced Cancer in Human Breast Cells MCF10A through Activation of Nrf2 Signaling Pathway

Zaid H. Maayah and Hesham M. Korashy. Department of Pharmacology & Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Purpose: Recent studies have established that metformin (MET), an oral hypoglycemic drug, possesses antioxidant activity and effective against different types of cancer in several carcinogen-induced animal models and cell lines. However, whether MET can protect against breast cancer has not been reported before. The overall objectives of the present study are to elucidate the potential chemoprevention effect of MET in non-cancerous human breast cells MCF10A model and explore the underlying mechanism(s) involved.
Methods: Non-cancerous human breast cell line MCF10A was utilized as a model for the study. The intensity of DNA adduct formation in MCF10A cells by DMBA was examined by measuring the mRNA expression levels of the Cytochrome P450 1A1 (CYP1A1), NAD(P)H:quinone oxidoreductase 1(NQO1) and the DNA repair genes, 8-oxoguanine DNA glycosylase (OGG1) and apurinic/apyrimidinic endonuclease1 (APE1) using real-time polymerase chain reaction. CYP1A1 and NQO1 protein levels were determined by Western blot analysis. The level of oxidative DNA damage was estimated by measuring the amount of 8-hydroxy-2′-deoxyguanosine (8-HDG). The involvement of aryl hydrocarbon receptor (AhR) and NF-E2-related factor 2 (Nrf2)-dependent mechanism were determined using the AhR and Nrf2-dependent luciferase reporter gene.
Results: Our results showed that DMBA-induced DNA adduct and damage was completely prevented by MET in a concentration-dependent manner as evidenced by decrease in CYP1A1, OGG1, and APE1 gene expression levels and the amount of 8-HDG level. Importantly, this was associated with a significant increase in NQO1 mRNA and protein levels. Mechanistically, the ability of MET to inhibit *CYP1A1* and to induce *NQO1* genes was strongly correlated with its ability to induce Nrf2/ARE- and inhibit AhR/XRE-dependent luciferase activity.
Conclusion: The present work provides the first evidence that MET inhibits the DMBA-mediated carcinogenicity by inhibiting the expression of CYP1A1 and induction of NQO1 through an AhR- and Nrf2-dependent mechanism.

94. Effect of Chronic Human Exposure to Environmental Heavy Metals in Mining Area on the Expression Profile of Oxidative Stress Genes

Ibraheem M. Attafi, Saleh A. Al-Bakheet and Hesham M. Korashy. Department of Pharmacology & Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Purpose: Living around polluted area is one of the most sources for exposure to environmental toxicants. Of these, heavy metals are widely used in foundries, mining, and manufacturing industries which exist in the environment either in a solid form as particulates or in a vapor form. Long term exposures and accumulation of heavy metals in the body may perturb oxidative stress genes and then increase the susceptibility to various diseases. The objective of the present study was to evaluate the long-term exposure to low level of heavy metals on gene expression of oxidative stress markers in the people living around mining area compared with people living in non-polluted area.
Methods: Sixty apparently healthy volunteers were divided to two groups; the first group consisted of 40 male residents in heavy metal-polluted area (exposed group), whereas the second groups consisted of 20 male residents in non-polluted area (control group). Specific criteria for selection were followed for both exposed and control groups through information given in the questionnaires. Whole blood concentrations for heavy metals, particularly lead, cadmium, and mercury, were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Total RNA was isolated using PAXgene Blood RNA kits. The mRNA levels of well-known heavy metals marker genes, NAD(P)H:quinone oxidoreductase 1 (NQO1), heme oxygenase 1(HO-1), and metallothionein 1 (MT-1), in exposed groups were compared with those in respective controls using real-time polymerase chain reaction (RT-PCR).
Results: Thirty-two (80%) volunteers from the exposed group showed a significant increase in heavy metals plasma concentrations as compared to control group. Among those exposed group, approximately 16 (37.5%) were highly polluted with lead with a mean concentrations of approximately 24 µg/L, whereas 10 (25%) and 9 (22.5%) subjects from exposed group were highly polluted with cadmium (2.5 µg/L) and mercury (1.65 µg/L), respectively. Importantly, quantitative RT-PCR results revealed significant inhibitions of all marker genes, in which lead-exposed group showed the highest inhibitory effect on NQO1 gene (60%), whereas cadmium-exposed group exhibited the highest inhibitory effect on MT mRNA expression by approximately 50%.
Conclusion: Exposure to environmental heavy metals differentially altered the expression of genes involved in oxidative stress, which could be used in risk assessment of an early preventative purpose and understand the mechanism of long-term exposure to heavy metals.

95. Regional Vulnerability to Oxygen-Glucose Deprivation in Hippocampal Slices may be a Function of Animal Age

Crystal C. Lalonde and John G. Mielke. School of Public Health and Health Systems, University of Waterloo, Waterloo, Canada

Purpose: The hippocampus is a structure in the temporal lobe that plays a critical role in the formation of many types of memory. Previous *in vivo* studies have shown that the hippocampus displays regional variability in the degree of injury observed following ischemic insult. In addition, many animal studies have found that increasing age, while not linearly related to the magnitude of ischemic damage, may worsen outcome. Given that hippocampal susceptibility to injury may vary by age and region, we decided to examine the effect and interaction of these variables within an *in vitro* model of ischemia that would be more amenable to subsequent cellular analyses.
Methods: Hippocampal slices were prepared from male, Sprague-Dawley rats of various ages, and were challenged with 15 minutes of oxygen-glucose deprivation (OGD) followed by a 3 hour recovery period. After this, the viability of either entire slices, or dissected sub-fields (i.e., CA1, CA3, dentate gyrus), were assessed by measuring changes in 2,3,5-triphenyltetrazolium chloride (TTC) metabolism. The reduction of TTC by mitochondria produces a reaction product within tissue that can be extracted and quantified with spectrophotometry.
Results: Slices were prepared from animals at four distinct points across the lifespan: pre-adolescence (3 weeks), late adolescence (7-8 weeks), young adult (25-36 weeks), and mature adult (60-63 weeks). Post-OGD metabolism of TTC was similar across age groups ($p = 0.86$). Within slices taken from

animals at 15 weeks of age, post-OGD TTC metabolism was significantly reduced in the CA sub-fields, but not in the dentate gyrus. In contrast, when the same comparison was completed using tissue from animals at 52 weeks of age, each sub-field displayed a significant reduction in metabolism of TTC.

Conclusions: Our results indicate that post-OGD metabolism of TTC does not differ among hippocampal slices prepared from animals between the ages of 3 weeks and 14 months. However, the reduced susceptibility of the dentate gyrus observed in slices from younger animals appears to be lost in tissue prepared from older animals. As a result, within the range examined, increasing age may not affect post-ischemic hippocampal viability in general, but may alter the response to injury within specific hippocampal regions.

96. Arginine and Glutamate Rich 1 is a Novel Protein Regulating Glucocorticoid Signalling

Lilia Magomedova, Stephane Angers and Carolyn L. Cummins. Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada

Purpose: The glucocorticoid receptor (GR) was the first member of the nuclear receptor (NR) family of transcription factors to be cloned. GR is expressed in virtually every tissue in the body, however, transcriptional regulation by GR is highly promoter and cell type dependent. GR transcriptional activation depends on its interactions with coactivators and corepressors. Most of the known NR coactivators and corepressors are ubiquitously expressed and interact with numerous NRs. How then is GR signalling cell and promoter specific? We hypothesized that novel protein-protein interactions underlie this selectivity. Using a high-throughput co-transfection assay we identified the protein arginine and glutamate rich 1 (ARGLU1) as a novel protein with no known function that strongly potentiates GR activity. Our objective is to uncover the mechanism by which ARGLU1 modulates GR activity.

Methods: GAL4-GR/UAS-luciferase reporter assays in HEK293 cells were used to map the critical domains important for ARGLU1-GR interaction. Stable HEK293 cell lines over-expressing Flag-tagged ARGLU1 and Flag-tagged GR were generated and the tagged proteins were affinity purified and analyzed by LC/MS. Primary mouse hepatocytes (C57Bl/6) were isolated and transfected with siControl or siARGLU1 using RNAiMAX Reagent. 48 hrs post-transfection, ligands were added to the cells for 4 hrs followed by RNA extraction. The glucose production assay was carried out 48 hrs post-siRNA transfection. ARGLU1 was knocked down in RAW264.7, as in primary macrophages.

Results: The C-terminal domain of ARGLU1 is responsible for the observed GRα coactivation. The ARGLU1 protein interaction network is composed of numerous spliceosomal proteins as well as, JMJD6, a recently identified, histone arginine demethylase. QPCR analysis revealed that following ARGLU1 knockdown, the induction of PEPCK and G6PC (two genes critical in the gluconeogenesis) were markedly reduced following dexamethasone (Dex) or corticosterone administration. Remarkably, glucose production was also significantly blunted in Dex-treated siARGLU1 cells compared to siControl. In RAW264.7 macrophages with siARGLU1 knockdown, we observed no change in the Dex-mediated basal suppression of IL-1β and TNFα expression.

Conclusion: ARGLU1 is a novel protein that strongly potentiates GR activity through its C-terminal domain. ARGLU1 is present in the spliceosomal complex, suggesting it plays a role in splicing in addition to being a GR coactivator. Finally, ARGLU1 was found to be critically important for Dex-induced gluconeogenic gene expression.

97. Carrageenan-Induced Hyperalgesia is Associated with Cortical EEG Slowing

Muffadal Shamshuddin, Raul Sanoja and Peter J. Soja. Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada

Purpose: Evidence exists that chronic pain in humans is associated with altered EEG activity toward slower frequency theta (θ) bandwidths (*Brain,* 2006, 1:55-64), a phenomenon referred to as thalamocortical dysrhythmia (TCD, *PNAS,* 1999, 96:15222-7). Intraplantar injection of the polysaccharide carrageenan (CAR) produces hyperalgesia lasting ~72hr (*J. Neuroscience*, 2000, 20:4680-5). Whether chronic hyperalgesia produces central sensitization that manifests itself as EEG slowing in the θ bandwidth is not known. The present study addressed this issue.

Methods: Mechanical (von Frey) and thermal

(Hargreave's test) sensory thresholds of the left and right hind paws were measured in male Sprague Dawley rats before (control, CON), and 2, 4, 24, 48, 72 hrs after intraplantar injections of CAR (1%, 100μL) or SAL (0.9%, 100 μL). In separate experiments performed under 1.5% isoflurane (ISO) anesthesia, the animals head was fixed to a stereotaxic frame and bilateral parietal cortical EEG activities were recorded around CAR injections. Heart rate, core temperature and O_2/CO_2 levels were maintained within normal limits. Ongoing EEG activity as well as evoked activity during mechanical (air-puff, 30psi, 10s) and thermal (Peltier contact probe, ramp 30-50°C, 10s on, 20s off) receptive field stimulation were quantified and expressed as mean ± S.E.M. Power spectra of cortical EEG during ISO experiments were calculated between 1-100Hz using a Fast Fourier transformation (FFT, 512 point). Frequency/power plots across standard EEG bandwidths were constructed.

Results: Behaviorally, rats injected with CAR, but not SAL developed an ipsilateral mechanical hyperalgesia as well as thermal hyperalgesia. Mechanical hyperalgesia remained for the entire observation period whereas thermal hyperalgesia lasted for a few hours. During ISO anesthesia, CON EEG activity consisted of a recurrent "burst-pause" waveform signature. Following CAR injection, it shifted to a pattern of repetitively occurring, large-amplitude slow waves, which was maintained throughout the remaining recording period. FFT analyses indicated that the predominant change in EEG activity was due to a significant, long-lasting increase in δ and θ bandwidth power; a phenomenon that is partly reminiscent of TCD reported in humans. Mechanical and thermal stimulation also evoked noticeable changes in EEG activity during CON, which were significantly enhanced after CAR (P<0.0001, Friedman's test).

Conclusions: CAR induces long-lasting mechanical and transient thermal hyperalgesia in rats that is associated with a sustained shift in EEG wave activity toward slower δ and θ bandwidths when compared to control baseline during ISO. EEG slowing described herein may constitute a valuable biomarker for the presence of central sensitization.

Acknowledgements: Supported by CIHR Muffadal Shamshuddin is an awardee of the National Summer Student Research Program Awards to Present Research Findings sponsored by GlaxoSmithKline Inc.

Clinical Sciences & Pharmacy Practice

98. Psychometric Analysis of the Multiple Mini-Interview as an Admissions Tool for the Leslie Dan Faculty of Pharmacy

Allan Choi, Linda MacKeigan, Andrea Cameron. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, ON, Canada

Background: Admissions interviews are used by most health professional schools in North America to assess personal qualities important to being a professional. The Leslie Dan Faculty of Pharmacy adopted the Multiple Mini-Interview (MMI) in 2010.
Objectives: To investigate the following psychometric properties of the MMI: 1) reliability 2) discriminant validity compared to other admission tools and 3) predictive validity with respect to Year 1 pharmacy performance.
Methods: 580 interviewees in 2010 consented to use of their admissions and academic data for research purposes. To examine MMI reliability, an intra-class coefficient (ICC) was calculated. Discriminant validity was assessed with Pearson r coefficients between pre-pharmacy average, Pharmacy College Admission Test Composite Score (PCAT comp), and MMI Score. To examine predictive validity, multiple regression analyses were conducted. Predictor variables were pre-pharmacy average, PCAT comp, MMI score, gender, and age. Criterion variables were first-year GPA and grades for three practice-oriented courses. MMI contribution to prediction of academic performance was assessed by the incremental R2 between regression models with and without the MMI.
Results: The MMI ICC was 0.73. Pearson r coefficients between MMI score and pre-pharmacy average and PCAT comp score were 0.12 and 0.11 respectively. Incremental R2 was 0.3% for first-year GPA, and 0.3%, 0.2% and 2.7% for each course.
Conclusion: The MMI is a reliable tool for assessing attributes of pharmacy applicants not captured by pre-pharmacy average and PCAT comp score. Due to the nature of the first-year curriculum, the MMI predicted academic performance in one course only whereas pre-pharmacy average and female sex consistently predicted all academic performance measures.

Acknowledgements: The author thanks Sandra Parna for providing admissions data and the GlaxoSmithKline for the National Summer Student Research Program Award. This project was funded by a CIHR Professional Student Research Award. The author declares that he is a member of the 2010 admissions cohort.

99. Benign Asymptomatic Transaminase Elevation Correlation with Adiponectin and Natural Killer Factor

Manuela G. Neuman[1], Yaakov Maor[1], Nir Hilzenrat[2]
[1]In Vitro Drug Safety and Biotechnology, and Department of Pharmacology and Toxicology, University of Toronto, ON, Canada, [2]Hepatogastroenterology, Sheba Medical Centre, Tel Aviv, Israel; [3]Gastroenterology, Jewish General Hospital, Montreal, QC, Canada.

Background: Patients with even mild elevations of alanine or aspartate aminotransferase might be in danger of using hepatotoxic medications such as acetaminophen or nonsteroidal anti-inflammatory drugs leading to oxidative stress. Screening allows many chronic hepatitis patients to be diagnosed early in the course of their disease.
Aim: Our aim was to profile natural killer factor (NFκB) and adiponectin in sera of patients with long-term benign transaminasemia and liver biopsies showing non-specific, non-diagnostic features of inflammation.
Methods: We recruited 18 patients with alanine aminotransferase (ALT) levels x 3–4 normal. The patients had been investigated for HCV, HBV, possible autoimmune or genetic hepato-biliary diseases, alcohol-non-alcoholic-hepatitis, drug or herbal-induced hepatotoxicities. We compared these patients with 30 healthy-controls that have normal ALT and with 30 HCV-controls with ALT levels x 3–4 normal. All 78 individuals have had their liver biopsies available (inflammation 0-1; fibrosis 0-1). We evaluated: NFκB (pg/mL).
Results: Patients with asymptomatic transanemia presented a significant higher level of NFκB 145.5 ± 25.5 ($p<0.05$) when compared to healthy-controls 32.5±14.5. HCV-patients have a higher level of NFκB 206.5 ± 28.0 ($p<0.05$) compared with the other 2 groups. Adiponectin levels show a significantly higher level compared both with healthy-controls and HCV.
Conclusion: We concluded that in patients with inexplicable ALT elevation, NFκB showed differences in sera that might be activating an expression of other oxidative-stress related proteins and proinflammatory cytokines. High levels of NFκB may influence the low but constant liver tissue inflammation. Adiponectin elevation can be related to bacterial intestinal translocation. Monitoring the correlation between the degree of the inflammation and the serum NFκB levels will improve the quality of patient follow-up and lead to future treatment modalities. Also, screening these patients early in their disease course leads to better treatment response, leading to prevention of cirrhosis.

100. An Adaptive Sequential Cohorts Design to Investigate the Safety, Tolerability and Pharmacokinetics of New Entities: An Emerging Trend in First-In-Man Clinical Trials

Mira Francis, Julie Basque, Eric Sicard and Marc Lefebvre. Algorithme Pharma Inc., Laval, Quebec, Canada

Purpose: To overcome the long-delays challenge in early stage clinical development, a novel approach was designed primarily for an adaptive assessment of the safety of new entities through overlapping single ascending doses (SAD) and multiple ascending doses (MAD) phases.
Methods: In a double-blinded design, the SAD phase is conducted in prescheduled cohorts receiving increasing doses projected from NOAEL, meanwhile, optional adaptive-cohorts run in parallel at intermediate doses where the dose level (1) should not exceed the dose evaluated in ongoing cohort (2) should not exceed the Maximum Tolerated Dose (MTD) if reached (3) should not exceed a predetermined cutoff dose and (4) is judged safe following review of data from prior doses. To further ensure subject's safety, a Sentinel approach is implemented at each dose. The MAD phase is initiated in parallel with the SAD phase only following favorable safety review of data from 3 Cohorts of the SAD. For the subsequent MAD cohorts, the dose level is confirmed based on safety data available from (1) completed cohorts in the SAD and (2) previous dose levels of the MAD. Each cohort can be conducted at the dose preselected or adjusted to an intermediate dose.
Results: Recently, this new approach was

successfully implemented during the conduct of multiple clinical programs for new drug molecules. In a clinical program for a new therapeutic peptide, 64 subjects were enrolled; 30 received single doses, 18 received multiple doses and 16 received placebo. The SAD was conducted over 10 weeks in 5 cohorts. The MAD was initiated 4 weeks following the start of the SAD, and was concluded within 6 weeks in 3 cohorts. Overall, the clinical program lasted over a short period of 2.5 months where the drug was well tolerated in healthy subjects. None of the 64 subjects was withdrawn for safety reasons. In comparison to the traditional first-in-man trials where the MAD phase is initiated following completion of the SAD phase at predefined dose levels, this adaptive approach offers the advantage of conducting both phases in parallel together with a possibility of investigating new dose levels in optional cohorts based on results observed in ongoing cohorts. Although this design demands a pre-trial implementation of restrictive start and stopping criteria to ensure subject's safety, both SAD and MAD phases could be completed simultaneously, allowing an expedited entry into Phase II.

Conclusion: This adaptive design significantly decreases the overall period of the early stage clinical assessment of new entities.

Pharmacokinetics & Pharmacodynamics

101. Pharmacokinetics and Biodistribution of Radiolabeled Hyperbranched Polyglycerol, a Novel Blood Pool Imaging Agent

Pavel Gershkovich[1], Katayoun Saatchi[1], Olena Sivak[1], Nikolaus Gelder[1], Donald E. Brooks[2], Kishor M. Wasan[1], Urs O. Häfeli[1]. [1]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada; [2]Centre for Blood Research, University of British Columbia, Vancouver, British Columbia, Canada

Purpose: To assess the pharmacokinetics, tissue distribution and plasma lipoprotein distribution of the novel blood pool imaging agent ^{67}Ga-labeled hyperbranched polyglycerol (^{67}Ga-HPGN)

Methods: ^{67}Ga-HPGN was administered as intravenous bolus (1.20±0.05 MBq of ^{67}Ga bound to 1 mg of HPGN in phosphate buffered saline pH 7.4) to male Sprague Dawley rats equipped with jugular vein catheters. Systemic blood (0.25 mL) was sampled 5 min pre-dose and 5, 15, 30 min post-dose, and then 1, 2, 4, 8, 24, 48 and 72 h post-dose. At 168 h post administration the animals were sacrificed and whole blood, heart, liver, right kidney, lungs, brain, spleen and quadriceps muscle harvested for biodistribution analysis. To study the plasma lipoprotein distribution the plasma was subjected to density-gradient ultracentrifugation. The radioactivity of blood samples, plasma fractions and collected tissues was analyzed using a gamma-counter.

Results: A two-compartment model was implemented for fitting the plasma concentration data ($\alpha = 0.45 \pm 0.18$ h^{-1}; $\beta = 0.02 \pm 0.002$ h^{-1}). The intravenous administration of ^{67}Ga-HPGN resulted in a long circulation time with a mean residency time (MRT) of 50.7 ± 5.2 h, a Vss of 918 ± 49 mL/kg and a CL of 18.2 ± 2.1 mL/h/kg. The radiolabelled compound was still detectable in the blood as late as 168 h post administration. Biodistribution results showed some uptake of ^{67}Ga-HPGN in liver, spleen and lung (20.84 ± 3.01 KBq/g, 27.46 ± 15.50 KBq/g and 38.04 ± 9.05 KBq/g, respectively). The lipoprotein distribution study showed the vast majority of ^{67}Ga-HPGN (>98%) in the lipoprotein-deficient plasma fraction.

Conclusions: The pharmacokinetic and biodistribution data suggest good *in vivo* stability of the ^{67}Ga-labeled polyglycerol and a slightly longer blood circulation time than red blood cells with disposition mostly into organs of the reticuloendothelial system. Thus the ^{67}Ga-HPGN radiopharmaceutical is the preferred choice for a blood pool imaging agent.

*Part of this work was presented at AAPS 2011 Annual Meeting and Exposition.

102. Vorinostat with Sustained Release and High Solubility in Poly(ethylene glycol)-bpoly(DL-lactic acid) Micelle Nanocarriers: Characterization and Effects on Pharmacokinetics in Rat Serum and Urine

Casey L. Sayre[1], Stephanie E. Martinez[1], Elham A. Mohamed[2,3], Mahasen M. Meshali[3], Connie M. Remsberg[2], Yunqi Zhao[4], Thanaa M. Borg[3], Abdel Monem M. Foda[3], Jody K. Takemoto[2], M. Laird Forrest[4], and Neal M. Davies[1] . [1]Faculty of Pharmacy, University of Manitoba, Winnipeg, MB, Canada [2]College of Pharmacy, Washington State

University, Pullman, WA, USA [3]Faculty of Pharmacy, Mansoura University, Mansoura, Egypt [4]Department of Pharmaceutical Chemistry, The University of Kansas, Simons Labs, Lawrence, Kansas, USA.

Purpose: The histone deacetylase inhibitor suberoylanilide hydroxamic acid, known as vorinostat, has promising potential as an anticancer agent. However, several difficulties present obstacles to the development of parenteral formulations. These include: low water solubility, low permeability, and suboptimal pharmacokinetics.
Methods: In this study, poly(ethylene glycol)-b-poly(DL-lactic acid) (PEG-b-PLA) nanomicelles of vorinostat at drug to carrier ratios of 1:10 and 1:15 were developed and characterized with respect to diameter, loading, and encapsulation efficiencies. Effects on vorinostat pharmacokinetics in rats were investigated after intravenous (10 mg/kg) and oral (50
mg/kg) administration compared to the respective controls of PEG400 solution and 2% methylcellulose suspension.
Results: The micelles showed high solubility (> 8 mg/ml), suitable diameter range (67395 nm), loading efficiency (9.93 ± 0.21% and 16.91 ± 1.19 %) and encapsulation efficiency (42.74 ± 1.67% and 73.29 ± 4.78%) for 1:10 and 1:15 nanomicelles, respectively. The micelles, particularly at 1:15 ratio, provided sustained release and improved pharmacokinetics characterized by a significant increase in serum half-life, area under curve, and mean residence time accompanied by significant reduction in renal and non-renal clearance.
Conclusion: Thus, PEG-b-PLA nanomicelles containing vorinostat may be suggested as a potential delivery system with improved disposition with potentially lower renal and hepatic toxicitiy. These findings warrant further clinical studies.

103. Assay Development, Pre-clinical Pharmacokinetics, and Pharmacodynamics of 3-methoxypterostilbene

Stephanie E. Martinez, Casey L. Sayre, and Neal M. Davies
Faculty of Pharmacy, University of Manitoba, Winnipeg, Canada

Purpose: To develop and validate an analytical assay to quantify 3-methoxypterostilbene in biological fluids, to evaluate the pharmacokinetics of 3-methoxypterostilbene in a rat model (administered intravenous and orally), and to determine the activity of 3-methoxypterostilbene in several pharmacodynamics models.
Methods: A novel and simple isocratic RP-HPLC method was developed to quantify 3-methoxyperostilbene in rat serum and urine. This method employed a Phenomenex® C18(2) column with UV detection at 327 nm. The mobile phase consisted of acetonitrile and water (62:38 v/v) with a flow rate of 1.05 mL/min. Male Sprague-Dawley rats were cannulated and dosed either intravenously with 3-methoxypterostilbene in PEG 600 (10 mg/kg) or orally with 3-methoxypterostilbene in PEG 600 (100 mg/kg). Serum and urine samples were collected over a 72h period post-dose. Antioxidant capacity, COX-1 and -2 inhibition, and α-glucosidase inhibition of 3-methoxypterostilbene were assessed via commercially available assay kits.
Results: The RP-HPLC method was successfully applied to a 3-methoxyptoerstilbene pre-clinical pharmacokinetics study. Serum and urine standard curves were both linear over 0.05-100 μg/mL. After intravenous and oral administration, 3-methoxypterostilbene was detected in both serum and urine as the parent compound and as a glucuronidated metabolite. 3-methoxypterostilbene possessed primarily concentration dependent antioxidant capacity and COX and α-glucosidase activities.
Conclusions: A novel, simple, sensitive, and isocratic RP-HPLC assay was developed for 3-methoxypterostilbene detection in biological fluids. A pre-clinical pharmacokinetic study of 3-methoxypterostilbene suggested that it is bioavailable and undergoes phase II metabolism. 3-methoxypterostilbene demonstrated *in vitro* concentration dependent antioxidant, COX inhibitory, and α-glucosidase inhibitory activities.

104. Tissue Distribution of Cyclosporine A Following Oral Administration of Cyclosporine A-loaded Polymeric Micelles to Rats: A Pilot Study

Ziyad Binkhathlan[1, 2], Dion R. Brocks[1], and Afsaneh Lavasanifar[1, 3]
[1] Faculty of Pharmacy and Pharmaceutical Sciences, Katz Group Centre for Pharmacy & Health Research, University of Alberta, Edmonton, Alberta, Canada.

[2] Nanomedicine Research Unit, Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[3] Department of Chemical and Material Engineering, University of Alberta, Edmonton, Alberta, Canada

Purpose: Previous studies have shown that polymeric micelles based on methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) can change the biodistribution pattern of the Cyclosporine A (CyA), reducing its accumulation in normal tissues and increasing its levels in blood, after a single intravenous dose to rats. The purpose of this study is to assess whether these polymeric micelles can have a similar effect on tissue distribution of CyA following oral administration.
Methods: Block copolymer of methoxy PEO-*b*-PCL (methoxy PEO and PCL of 5000 and 13000 g/mol, respectively) was synthesized. CyA was physically encapsulated in PEO-*b*-PCL micelles using co-solvent evaporation method. The control formulation of CyA was the commercially available formulation (Sandimmune®). Six Sprague-Dawley (290-320 g) rats were used in this study (3 rats/group). CyA was orally administered as a single dose of 10 mg/kg. Heart, spleen, liver, kidney, as well as samples of blood and plasma were collected six hours after the drug administration. Samples were assayed for CyA concentrations using an LC/MS method. The data were analyzed for statistical significance by unpaired Student's *t*-test ($\alpha = 0.05$).
Results: CyA concentrations were higher in all tissue as well as blood and plasma samples after administration of micelle formulation compared to Sandimmune® (Table 1). However, it reached statistical significance ($p < 0.05$) only in liver and spleen tissues. Blood to plasma ratios were comparable for both formulations (1.57 *versus* 1.82 for Sandimmune® and micelles, respectively). Tissue to plasma ratios (K_p) for CyA were also comparable for both formulations except for heart and spleen tissues, where they tended to have higher K_p values after administration of the polymeric micellar formulation.
Conclusion: The results suggest that encapsulation of CyA inside polymeric micelles may alter the tissue distribution of CyA following oral administration to rats. Further study is needed to permit a definitive conclusion.

Table 1. Plasma, blood and tissue concentration of CyA six hours after oral administration of CyA-loaded polymeric micelles or Sandimmune® to Sprague-Dawley rats (n = 3/group)

Specimen	Sandimmune®	PEO-*b*-PCL micelles
Plasma (μg/mL)	0.28 ± 0.11	0.62 ± 0.19
Blood (μg/mL)	0.43 ± 0.19	1.15 ± 0.58
Liver (μg/g)	44.7 ± 16.1	105.2 ± 23.8*
Kidney (μg/g)	18.6 ± 6.5	46.7 ± 20.0
Heart (μg/g)	13.3 ± 7.6	39.7 ± 15.7
Spleen (μg/g)	30.2 ± 14.4	88.0 ± 23.6*
*Denotes significant difference ($p < 0.05$) between groups.		

105. Effect of Inflammation on Maternal and Fetal Disposition of Lopinavir in Rats

Vanja Petrovic and Micheline Piquette-Miller. Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada

Purpose: The protease inhibitor lopinavir (LPV) is widely used for clinical antiretroviral therapy in pregnant women and for preventing the mother-to-child transmission (MTCT) of the virus. Maintenance of optimal LPV concentration is essential for effective therapy and MTCT prevention, but little is known about the effects of inflammatory mediators on the pharmacokinetics of this protease inhibitor and drug transporter substrate. While inflammation is known to impose changes in the expression and activity of drug transporters, there is a scarcity of data regarding the impact of inflammatory stimuli on drug transporter expression and substrate disposition during pregnancy. We examined the effect of inflammation on key maternal drug transporters and maternal and fetal disposition of lopinavir.
Methods: The viral mimetic, polyinosinic: polycytidylic acid (poly I:C, 5.0 mg/kg i.p.), was used to induce acute inflammation in pregnant Sprague-Dawley rats on gestational day 17-18 (with saline vehicle controls). At 24 hours post-injection, all rats were intravenously administered LPV (10 mg/kg). Maternal plasma, liver and placentas along with the fetal units were collected at several time points after LPV administration. LPV concentrations were determined by LC-MS/MS. Expression of transporters was measured via real-time qPCR.
Results: Overall, poly I:C-induced inflammation imposed significant downregulation ($p<0.05$) in the expression of several important drug transporters in placenta and liver of pregnant rats, mainly mdr1a,

mdr1b and mrp2, as well as cyp3a2. There was a significant increase in maternal LPV plasma concentration and AUC in rats infected with poly I:C (p<0.01). However, the hepatic, placental, and fetal LPV concentrations and AUCs did not exhibit the same trend, likely due to differences in plasma protein binding, suggesting the possibility of underexposure to LPV during an inflammatory state.
Conclusion: Since the majority of commonly used and clinically important protease inhibitors are known to be ABC transporter substrates and are highly protein bound, viral exposure and the subsequent inflammation-mediated changes in transporter expression and plasma protein binding could affect maternal disposition, fetal exposure and clinical outcomes.

106. Effect of a Surgery-induced Inflammatory Response on the Brain Distribution of Morphine and its Glucuronidated Metabolites

Kathryn Landry[1], Lisa. C. Julien[3], Lekha L. Sleno[4], Mark Issa[1], Derek J. Roberts[5], Richard Hall[2,3] and Kerry Goralski[1,2]. [1]College of Pharmacy and [2]Dept. of Pharmacology, Dalhousie University, Halifax, NS, [3]Dept. of Anaesthesia, QE2 Health Sciences Centre, Capital Health District Authority, Halifax, NS, [4]Département de Chimie, Université du Québec à Montréal and [5]Departments of Surgery, Critical Care Medicine, and Community Health Sciences, University of Calgary and the Foothills Medical Centre, Calgary, AB

Purpose: To determine the effect of a surgery-induced inflammatory response on the central nervous system (CNS) distribution of morphine and morphine 3-(M3G) and 6-glucuronide (M6G) in adults. We hypothesized that surgically-induced inflammation would increase the cerebrospinal fluid (CSF) concentration of morphine, M3G, and M6G through a temporary reduction in blood-brain barrier (BBB) drug efflux transporter function.
Methods: Blood and CSF samples were collected from patients before, during, and after undergoing elective aortic surgery. Patients were divided into two groups according to their need for cardiopulmonary bypass (CPB) and the expected severity of their resultant inflammatory response: 1) those undergoing percutaneous aortic stent graft placement or receiving thoracotomy with aortic graft insertion and 2) those receiving thoracotomy with aortic graft insertion during CPB. Plasma and CSF were analyzed for morphine, M3G, M6G, cytokines (IL-1α, Il-1b, IL-2, IL-4, IL-6, IL-10, IL-12, IFNγ and TNFα), and albumin.
Results: To date, 25 of the 30 required patients have been enrolled in the study: 13 patients in the non-CPB group and 12 patients in the CPB group. Cytokine analyses have been completed for 18 of these patients. IL-6 was the most sensitive indicator of the inflammatory response to surgery, which was more profound in the circulation than in CSF. Moreover, the degree of this response was significantly greater in the CPB versus the non-CPB group ($P < 0.05$). CSF levels of albumin remained in normal range, suggesting that the physical BBB remained intact. Morphine and metabolite analyses have been completed in 13 patients demonstrating that morphine and its glucuronidated metabolites are measurable in CSF.
Conclusion: Completion of morphine and metabolite analyses will allow us to determine if the heightened systemic inflammatory response among patients that received CPB alters the CSF distribution of morphine and its metabolites compared to those that did not undergo CPB. These findings will allow us to determine whether the degree of the systemic inflammatory insult correlates with changes in CNS drug disposition. They will also assist in further elucidating the mechanism of inflammation-induced changes in drug disposition and altered drug responses in humans.
Acknowledgements: We thank the Heart and Stroke Foundation of Nova Scotia, the Pharmacy Endowment Fund and the Canadian Anaesthesiologists' Society for providing funding for this project. Kathryn Landry is an awardee of the National Summer Student Research Program Awards to Present Research Findings sponsored by GlaxoSmithKline Inc.

107. Nonparametric Superposition to Predict Ropinirole Pharmacokinetics Data at Steady-state using Single-dose Data

Zia R. Tayab and Juan He. BioPharma Services Inc., Toronto, ON, Canada

Purpose: The current EMA bioequivalence guideline states that steady-state studies are required for drugs which are extended-modified release, or if a single dose study cannot be conducted in healthy volunteers due to tolerability reasons, and a single

dose study is not feasible in patients. Ropinirole extended release tablets is known to fall into this category. The prediction of ropinirole steady-state levels using single dose data will support the development of an optimal steady-state design.

Methods: The data from four different single dose studies evaluating the bioequivalence of ropinirole under fasting conditions was pooled together. Each study evaluated the pharmacokinetics of Requip® XL 2 mg, in normal healthy male and female volunteers. The observed steady-state data from one bioequivalence study with 8 mg dose was used to confirm the results of the simulation. The steady-state data was simulated with the single dose data using the nonparametric superposition tool in WinNonlin® version 5.2.1 (Pharsight corporation. Cary, North Carolina, USA). The resulting simulated data was correlated to the observed steady-state ropinirole data, to determine how well the predicted data agrees with the observed data. In order to account for the differences in dose between the single dose data (2 mg) and the multiple dose data (8 mg), the multiple dose data was dose normalized considering that ropinirole is known to exhibit linear pharmacokinetics over the therapeutic dose range (2 mg to 8 mg).

Results: Using single-dose ropinirole data, the levels at steady-state were able to be predicted. The predicted AUC_{tau} and C_{max} at steady-state were 35405 pg/mL*hr and 1366 pg/mL, respectively. The dose normalized observed steady state AUC_{tau} and C_{max} parameters were 36739 pg/mL*hr and 1571 pg/mL, respectively.

Conclusions: A method to predict the steady-state levels of ropinirole using single dose data has been developed. Having *a priori* knowledge of the expected levels of ropinirole at steady-state based on single-dose data may be an additional beneficial tool in developing an optimal study design for evaluating a test product's bioequivalence at steady-state.

108. Prediction of Flow Effects on Intestinal (F_I) and Systemic (F_{sys}) Availability in PBPK Intestine Models: The Traditional (TM), Segregated Flow (SFM) and Q_{Gut} Models

Edwin C.Y. Chow and K. Sandy Pang. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Canada

Purpose: Compartmental models are no longer adequate to address effects of permeability barriers, intestinal and liver transporters and enzymes, and sequential metabolism within the intestine and liver during oral drug absorption, when intestinal removal is substantial relative to the liver and when distinctively different extents of alteration occur after induction/inhibition of the same intestinal and hepatic enzyme or transporter. Over the past decades, there has been substantial development towards physiologically-relevant PBPK intestinal models to inter-relate intestinal transporters and enzymes and blood flow to appraise their influence on intestinal (F_I), liver (F_H) and oral systemic (F_{sys} or $F_{abs}F_IF_H$) bioavailability. Thus, we re-examined three PBPK models [traditional model (TM), Q_{gut} model, and segregated flow model (SFM)] in which the fractional, intestinal flow (f_Q) to the enterocyte is 100%, 50% and 10%, respectively, to appraise how the enterocyte blood flow affects F_I and F_{sys} using simulations.

Method: The equation developed by Sun and Pang (Pharm. Res., 2010) on the area under the curve (AUC) after oral and intravenous drug administration was used. Simulations were performed upon varying the intestinal metabolic ($CL_{int,met1,I}$) or secretory ($CL_{int,sec,I}$) intrinsic clearances (0 to 10,000 ml/min), blood flow to the enterocyte region (620 ml/min for TM, 310 ml/min for Q_{gut}, and 62 ml/min for SFM), the basolateral influx (CL^{I}_{d1}) or efflux (CL^{I}_{d2}) transport clearances, and fraction absorbed (F_{abs}).

Result: Our result showed that the $CL_{int,met1,I}$ strongly influenced F_I, F_{sys}, the fractional contribution by the intestine as well as residual contribution by the liver. The role of $CL_{int,sec,I}$ was modest due to drug reabsorption. Induction or inhibition of drugs of lower $CL_{int,met1,I}$ (< 500 ml/min) greatly shifted values of F_I, more so for SFM than for TM. Increases in CL^{I}_{d1} and decreases in CL^{I}_{d2} led to reduced F_I. More importantly, F_I and F_{sys} were found much affected by given enterocyte flow rates, showing TM > Q_{Gut}, and lowest for SFM. Literature survey revealed that f_Q estimates (2.4 to 20% and 43% for midazolam) were closer to designated flow rates from the SFM and Q_{Gut} model, and fits to data pointed to the superiority of the SFM over the TM.

Conclusion: The development of these PBPK models has led to a refinement in predicting drug and metabolite profiles due to the ability of the models to describe individual transport and

metabolic processes in the intestine and liver.

109. Induction of Mdr1/MDR1 and P-glycoprotein in Rat (RBE4) and Human (hCMEC/D3) Brain Microvessel Endothelial Cell Lines and Isolated Rat Brain Capillaries by Vitamin D Receptor (VDR) Ligands

Matthew R. Durk[1], Gary N.Y. Chan[1], Christopher Campos[2], Anna K.Y. Fung[1], John Peart[2], Edwin C.Y. Chow[1], Eason Lee[2], Ronald E. Cannon[2], Reina Bendayan[1], David S. Miller[2], and K. Sandy Pang[1]. [1]Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada; [2]Laboratory of Toxicology and Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, U.S.A.

Purpose: The effects of vitamin D Receptor (VDR) ligands, 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] and lithocholic acid acetate (LCAa) on Mdr1a/Mdr1b and P-gp expression and function were appraised in rat (RBE4) and human (hCMEC/D3) brain microvessel endothelial cell lines and isolated rat brain capillaries *in vitro* for comparison to previous observations in mouse that VDR elevated brain Mdr1a mRNA and P-gp expression and function *in vivo*[1,2].
Methods: RBE4 and hCMEC/D3 cell lines were treated with VDR ligands, 1,25$(OH)_2D_3$ (10 to 200 nM) and lithocholic acid acetate, LCAa (1 to 20 □M) for 1 or 3 days. Protein and mRNA expression levels of VDR, MDR1/Mdr1a/Mdr1b/P-gp, and CYP24/Cyp24 were determined by immunoblotting and qPCR, respectively, whereas P-gp function was assessed by the extents of cellular accumulation of rhodamine 6G, a P-gp substrate. Freshly prepared rat brain capillaries, isolated from cortical, grey matter, were treated immediately with 1,25$(OH)_2D_3$ (1 to 100 nM) for 4 or 24 h. In these preparations, P-gp protein was assessed by immunoblotting and P-gp function, as the efflux into lumen of the fluorescent, P-gp substrate, [N-ε(4-nitrobenzofurazan-7-yl)-D-lys[8]]-cyclosporine A (NBD-CSA).
Results: In RBE4 cells, Mdr1b mRNA was induced in a dose-dependent manner after 1,25$(OH)_2D_3$ treatment for 1 and 3 days. P-gp protein increased significantly (2.5-fold) following the 3-day treatment with 100 nM 1,25$(OH)_2D_3$, and concomitantly, a 40% reduction in rhodamine 6G (R6G) accumulation was observed. Similar concentration-dependent induction patterns were observed following treatment with LCAa, which increased the mRNA expression of Mdr1a but not Mdr1b and P-gp protein. In hCMEC/D3 cells, MDR1 mRNA was induced by 40% after 3 days of treatment with 100 nM 1,25$(OH)_2D_3$, and was accompanied by a 3.25-fold increase in P-gp protein expression and 30% reduction in R6G accumulation. Incubation of isolated rat brain capillaries with 10 nM 1,25$(OH)_2D_3$ for 4 h resulted in a 4-fold induction of P-gp protein and increased the intraluminal efflux of NBD-CSA (~ 30%) at 4 and 24 h after treatment with 10 nM 1,25$(OH)_2D_3$.
Conclusion: This study demonstrated that MDR1/Mdr1 is upregulated by VDR ligands, 1,25$(OH)_2D_3$ and LCAa, at the cellular level in rodent and human brain microvascular endothelium, and in isolated rat brain capillaries, increasing both P-gp expression and function.

References:

[1] Chow et al. 2011 J Pharmacol Exp Ther. Jun;337:846-59.
[2] Durk et al. 2011 AAPS NBC Poster #T2103

Pharmaceutical & Analytical Chemistry

110. Chemical Reactivity and Biological Activity of Dihydro-1,4-dithiin Tetraoxides

Jordan D. Nash[a], Kim Madec[a], A. David Brewer[b], Brian B. Hasinoff[a], Gaik-Lean Chee[a,*]. [a]Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada; [b]Chemtura Canada Co./Cie, Guelph, Ontario, Canada.. *Corresponding author. E-mail: lean_chee@umanitoba.ca.

Purpose: Dihydro-1,4-dithiin tetraoxide is a ring system found in a number of biologically active compounds including dimethipin, a commercial plant growth regulant. Although the 1,4-α,β-unsaturated sulfone system in the ring has been suggested to be electrophilic, the reactivity of the system has not been carefully examined or correlated to the reported biological activities. In this study, we synthesized and investigated the reactivity as well as the biological activity of eight dithiin compounds unsubstituted, or mono- and di-substituted with simple inductively electron donating groups (EDG) or electron withdrawing groups

(EWG) at the α,β-unsaturated bond.

Methods: The reactivities of dithiin derivatives towards biologically important nucleophilic groups were examined at pH 7.4 using UV-vis, fluorescence, and ^{1}H-NMR spectroscopic techniques. The compounds were tested in a human K562 leukemia cell growth inhibition assay for their cytotoxic effects. Their ability to inhibit the catalytic activity of DNA topoisomerase IIα was examined in an enzyme-catalyzed kinetoplast DNA decatenation assay.

Results: The substitution patterns at the α,β-unsaturated bond of dithiin tetraoxides exhibited the following order of reactivity towards sulfhydryl nucleophiles: mono- or di-EWD ≈ unsubstituted > mono-EDG >> di-EDG. Dithiins with the first three substitution patterns reacted with molecules containing free sulfhydryl groups such as glutathione and cysteine, but did not react with amino, carboxylic, phenolic, and phosphate functional groups. Dithiins with di-EDG such as dimethipin did not react with any of the tested nucleophiles. The relative potencies of these compounds in the inhibition of K562 cell growth and topoisomerase IIα catalytic activity were found to correlate highly to their relative reactivity towards sulfhydryl groups.

Conclusions: The results suggest that reactive dithiins may exert their cytotoxicity by reacting with free sulfhydryl groups of biomolecules. Consistent with this view, the inhibitory activity of these reactive dithiins on topoisomerase IIα may be due to their ability to react with critical free cysteine sulfhydryl groups on the enzyme.

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

111. Discovery of Nonpeptidic Small Molecules Mimicking Protein Recognition Surfaces

Lianhu Wei,[1] Liyakat Fatima,[1] Meena Purohit,[1,2] Angelica M. Bello,[1,3] Beata Majchrzak-Kita,[1,4] Eleanor N. Fish,[1,4,5] Lakshmi P. Kotra[1,3,6]*. [1]Center for Molecular Design and Preformulations, University Health Network, Toronto, ON, Canada; [2]Birla Institute of Technology & Science, Pilani, India; [3]Leslie Dan Faculty of Pharmacy, University of Toronto, ON; [4]Division of Cell & Molecular Biology, Toronto General Research Institute, Toronto, ON; [5]Faculty of Medicine, University of Toronto, Toronto, Ontario; [6]McLaughlin Center of Molecular Medicine, University of Toronto, Toronto, Ontario, Canada.

Purpose: In a variety of disease processes, protein-protein interactions are a very important component and modulation of such interactions between the two partner molecules can be used as a target for new drug design to mitigate the pathologic process. In this context, small molecules to modulate the protein-protein interactions are seen as an ideal intervention strategy. Typically, protein surfaces are very large and hotspot regions are attributed with certain functional importance, either to bind to a partner protein or elicit a certain response. Here, we disclose the discovery of small molecules targeting one of the hot spot regions, IRRP-I on the surface of interferon using *in silico* screening, and discuss the significance of this region for molecular mimicry.

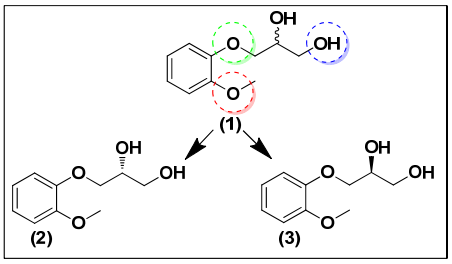
Methods: Three hotspot regions were identified to be responsible for the protein-protein interactions and the transfer of functional information between type I interferon receptor (IFNAR) and one of its ligand, interferon-α2a. A variety of small molecule structural databases were screened for potential small molecule mimics that could replicate the features of the one of the hotspot regions, IRRP-I surface using *in silico* chemical database search. Compound hits were further enriched with filters followed by visual inspection leading to the selection of the hits.

Results: Considering the synthetic and development feasibility, nine hits were selected for *in vitro* screening. Among these compounds KF-52, KF-53, and KF-59 showed promising results that these molecules can compete with the binding of interferon at its receptor.

Conclusions: The inhibition of the interferon activity shown by the hits selected from *in silico* screening suggested that the hits are binding at the receptor as intended. Mimicry of protein-protein interactions at the interface surface requires insight into the exact interaction points. Details of the *in silico* screening, hit discovery and their characterizations will be presented.

112. Effect of Chirality on Neurite Outgrowth of Sensory Neurons using Phenoxy Propanediol Derivatives

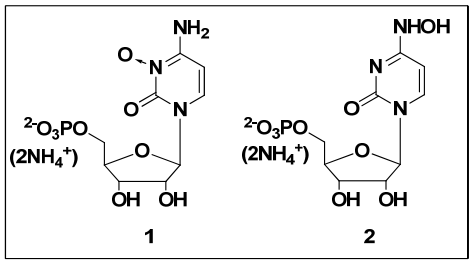
Meena K. Purohit,[1,3] Nigel A. Calcutt,[5] Paul Fernyhough[4] and Lakshmi P. Kotra[1,2,*]. [1]Center for Molecular Design and Preformulations, University Health Network, Toronto, ON; [2]Leslie Dan Faculty of Pharmacy, University of Toronto, ON; [3]Birla Institute of Technology & Science, Pilani, India; [4]Dept of Pharmacology & Therapeutics, University of Manitoba, Winnipeg, MB; [5]Division of Neurodegenerative Disorders, St Boniface Hospital Research Centre, Winnipeg, MB; [5]Dept of Pathology, UCSD, La Jolla, California, USA.



Purpose: Diabetic neuropathy is the most common complication of diabetes mellitus. Nerve biopsies from diabetic patients show several morphological and structural changes such as microangiopathy, axonal degeneration, and segmental demyelination are seen. Axonal degeneration typically occurs at the most distal end of the axon and the capacity of peripheral nerve to regenerate is impaired. There is a need of a small molecule drugs which can improve sensory axon sprouting and/or regeneration leading to the prevention and/or reversal of neurodegeneration in diabetic neuropathy.
Method: A library of 600 drug-like compounds was screened in-vitro for their ability to enhance axonal regeneration in cultures of adult rat sensory neurons in an attempt to indentify small molecule drugs. This high throughput screening led to the identification of guaifenesin, a phenoxypropyl derivative. A number of derivatives of this molecule were synthesized in order to dissect the responsible structural elements for the neurite growth activity and were screened for their axonal growth activity.
Results: A phenoxy propanediol derivative, (±)-guaifenesin (**1**)—a common component in various cough preparations—was identified as a potential agent to enhance axonal outgrowth. Initially racemate of compound (**1**) was tested for its axonal growth activity. Along with several phenoxy propanediol derivatives of compound (**1**), its enantiomers were synthesized and tested. Compound (**2**), an *R*-enantiomer of (**1**), exhibited very high potency in vitro at 0.01 μM concentration leading to over 3 fold neurite axonal outgrowth. None of the modifications incorporated onto the core structure of phenoxypropane led to higher potency.
Conclusion: Chirality of the phenoxypropane diol molecules is an important consideration for the neurite outgrowth, and this implies there may be a receptor or enzyme involved for this process. Further studies are required to establish the role of receptors.

113. N^3- and N^4-CMP Derivatives as Orotidine-5'-monophosphate Decarboxylase (ODCase) Inhibitors

Meena K. Purohit,[1,2] Ewa Poduch,[1] Lianhu Wei,[1] Ian Crandall,[6,7] Emil F. Pai[3,4,5] and Lakshmi P. Kotra[1,2,3,7*]. [1]Center for Molecular Design and Preformulations, Toronto General Hospital, Toronto, ON, [2]Institute of Technology and Science, Pilani, India, [3]Departments of Pharmaceutical Sciences and Chemistry, University of Toronto, [4]Ontario Cancer Institute, Princess Margaret Hospital, Toronto, ON, [5]Departments of Medical Biophysics, Biochemistry, and Molecular and Medical Genetics, Toronto, ON, [6]Department of Medicine, UHN-Toronto General Hospital and the University of Toronto and McLaughlin-Rotman Center/UHN, [7]McLaughlin Center for Molecular Medicine, University of Toronto, Toronto, Ontario, Canada.



Purpose: ODCase has been a subject of intense investigation over the past three decades due to its biochemical significance. Several C-6 substituted UMP derivatives were developed as potent ODCase inhibitors and their interactions with the active site of ODCase were studied comprehensively. As compared to UMP, cytidine-5'- monophosphate (CMP) is a weak inhibitor of ODCase and binds to ODCase in an unconventional conformation. Here

we reveal for the first time, two novel CMP derivatives that bind to ODCase with high potency and engage in productive interactions with the ODCase active site.

Methods: Compounds (**1**) and (**2**) were synthesized and were characterized to confirm the location of the oxidation. Enzymatic assays were performed at 37 °C or 55 °C using an isothermal titration calorimetric assay developed in house. CMP derivatives and ODCase were crystallized with human ODCase by using hanging drop technique.

Results: CMP is a weak ODCase inhibitor with a K_i of 1,200±700 and 1,400±100 μM against *Methanobacterium thermoautotrophicum* and human ODCases, respectively. Whereas compound (**1**) reversibly inhibits *M. thermoautotrophicum*, human and *Plasmodium falciparum* ODCases with a K_i of 11.1±0.4, 28.3±1.5, and 22.1±3.2 μM, respectively. Compound (**2**) was an inhibitor at low micromolar concentration as well. In the co-crystal structure of compound (**1**) bound to ODCase, a potential rearrangement in the nucleic base portion was observed.

Conclusion: Derivatization of the nucleic base within CMP improves the inhibitory potential of this class of molecules over 100 folds. The details of medicinal chemistry, structural biology and antiplasmodial activities of nucleic base derivatized CMP derivatives will be presented.

114. All-or-none Membrane Permeabilization by Fungicidal Lipopeptides from *Bacillus Subtilis* QST 713

Hiren Patel[1], Clemens Tscheka[2], Katarina Edwards[3], Goran Karlsson[3] and Heiko Heerklotz[1]. [1]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada; [2]Dept. of Pharmacy, University of the Saarland, Campus, Geb., Saarbrücken, Germany; [3]Dept. of Physical and Analytical Chemistry, Uppsala University, Uppsala, Sweden.

Purpose: The fungicidal activity of *Bacillus subtilis* QST713, based mainly on the production of cyclic lipopeptides of the fengycin (FEs), surfactin, and iturin families, has been utilized for the highly effective and environmentally safe protection of crops against a variety of pathogens. The mixed population of native FEs forms micelles which solubilize individual FEs such as agrastatin 1 (AS1) that are otherwise rather insoluble on their own but promote the membrane permeabilizing activity of the mixture.

Methodology: Membrane leakage and lysis are the most fundamental and practically important effects on membranes; our recent success in introducing a new, greatly improved version of the classic vesicle leakage assay (Patel et al., Soft Matter 2009) gives me a very substantial advantage for this work. Using biexponential fluorescence decay parameters instead of simple intensity values, we can track the amounts and local concentrations of entrapped and leaked-out dye simultaneously and see whether leakage occurs through specific pores or transient, homogeneously distributed membrane defects.

Results: Fluorescence lifetime-based calcein efflux measurements, isothermal titration calorimetry and electron microscopy show that these FEs show a unique scenario of membrane permeabilization. Poor miscibility of FEs with lipid induces stable, long-lived pores in 10% of the vesicles at only ≈ 1 uM free FE and in 15% of the vesicles at 10 uM.

Conclusion: We explain why this all-or-none leakage accounts for the killing of virtually all fungi whereas the same extent of graded leakage would likely be biologically irrelevant. Then, crystallization of AS1 and micellization of plipastatins cause a cut-off in leakage at 15% that might regulate the biological activity of FEs, protecting *Bacillus* and plant membranes. The fact that FE micelles solubilize only about 10 mol-% fluid lipid resembles the behavior of detergent resistance.

115. Effect of Nicotine on Dopamine Levels in Presence of Oligoelements in Brain Regions of Young Rats

David Calderon Guzman[1], Ernestina Hernandez Garcia[2], Francisca Trujillo Jimenez[2], Gerardo Barragan Mejia[1], Hugo Juarez Olguin[2], Norma Osnaya Brizuela[3]. [1]Laboratory of Neurochemistry, National Institute of Pediatrics, Mexico, [2]Laboratory of Pharmacology, Institute of Pediatrics, Mexico, [3]Laboratory of Neuromorfometry. Mexico.

Aim: The purpose of this study was to measure the effect of nicotine on dopamine levels of young rats in the presence of oligoelements.

Methods: Male Wistar rats (weight 80g) were treated with single and repeated doses of nicotine and/or oligoelements as follows: group 1(control) NaCl 0.9%; group 2, nicotine (1mg/kg); group 3,

oligoelements (50µl/rat); and group 4, nicotine (1mg/kg) + oligoelements (50µl/rat). All drugs were administered intraperitoneally for 4 days. Blood for dextrostis was obtained from all the animals. Samples of the brain regions (cortex, hemispheres and cerebellum + medulla oblongata) of each rat were obtained and used to measure the concentrations of dopamine levels using fluorescence methods, previously validated.

Results: The levels of glucose increased in blood of rats treated with nicotine and oligoelements ($p<0.05$). The levels of dopamine decreased in cortex and hemispheres, but increased in cerebellum/medulla and oblongata regions of rats treated with both compounds ($p<0.05$).

Conclusions: Nicotine and oligoelements are associated with increase in the level of glucose, an effect that was more pronounced in the group treated with both drugs. Reduction of oxidative stress and dopamine metabolism may be involved in these effects.

116. Starting a Revolution – Powder Flow Characteristics and Measurement

Daniel C. Alsmeyer, PhD and Ian Buxton. Apotex Inc., Toronto, ON.

The Revolution Powder Analyzer manufactured by Mercury Scientific Inc. is an instrument designed to assess the flow properties of powders and granular materials. Powder flowability is defined as the ease with which a powder will flow under a specified set of conditions. Some of these conditions include: the pressure on the powder, the humidity of the air around the powder, and the equipment the powder is flowing through or from. This presentation reviews the utility of some of the measurement characteristics and how the data is used to predict manufacturing powder flow concerns.

The Revolution instrument functions by rotating a powdered sample in a cylindrical drum and viewing the material as it builds and cascades. A digital camera records images of the powder during the rotation. During analysis, the powder sample flows upward until sufficient energy is collected that the material "breaks" or cascades downward in an avalanche. This process is shown in Figure 1.

Figure 1 – Revolution powder avalanche images

The instrument uses various algorithms to interpret the behavior of the powder from images captured during the rotation. The calculated parameters represent aspects of the powder's quality and process flowability.

The angle, height, and frequency of avalanche breaks are indicative of the flow characteristics of the powder material. The imaging software also assesses the energy in the sample before, during, and after the avalanche breaks. In general, the lower the avalanche time and energy, the better the powder will flow in manufacturing equipment. While the vendor recommends sample sizes of around 100 cc, an alternate drum cylinder permits smaller volumes to be analyzed. Overall analysis time was determined to be less than 30 minutes.

Powder samples were analyzed and correlated to manufacturing capability to build a library of experience. The established library allows quick prediction of flow capability in the manufacturing environment with a minimum of powder material during the drug development process. The resultant Revolution Powder Analyzer information can be used to quickly determine and ensure good flowability during development, well before the material is brought to the manufacturing floor.

117. Porphyrin Complexation: An Approach in Porphyria Therapy

Bolanle Akinwumi and Alan McIntosh, Faculty of Pharmacy, University of Manitoba, Winnipeg, MB

Purpose: Porphyria is a rare metabolic disease which occurs as a result of accumulation of porphyrins due to specific enzyme deficiency in the biosynthetic pathway of heme. Chloroquine is currently used in the treatment of cutaneous porphyria. The primary focus of this study is to explore the molecular complexation mechanism of chloroquine binding with protoporphyrin IX (PPIX). As a treatment option for chronic cutaneous porphyrias, in particular, the binding mechanism is poorly understood. Previous studies have focused on the complexation mechanism in aqueous media

which is probably inappropriate because of the hydrophobic nature of both molecules. We propose a study of complex formation *in vitro* between excess chloroquine with PPIX in two hydrophobic media: 50:50 acetone / dichloromethane mixed-solvent system; and aqueous micellar dispersions of the nonionic detergent Triton X-100^{R}. The aim of this research is to determine the dissociation constants for the complex formation between PPIX and chloroquine as well as related compounds and to identify the structural features that are important for the binding interaction.

Methods: Optical absorption difference spectroscopy has been utilized to measure complex formation between PPIX and chloroquine in the above hydrophobic media. Complexes of other acceptor molecules were also studied including molecules that are structurally related to chloroquine. Benesi-Hildebrand plot and Hill plot analyses were utilized for the determination of the equilibrium dissociation constants (K_D) of the weak and the strong complexes respectively. The analysis was carried out for the highly sensitive porphyrin Q-bands in the PPIX visible absorption spectrum.

Results: Based on their K_D±SEM values, the acceptor molecules studied were broadly classified into weak association molecules: duroquinone (102.4±18.2mM) and quinine (43.3±1.7mM), and strong association molecules: chloroquine (100.4±39.4μM), amodiaquine (378.8±30.2 μM), quinacrine (108.6±23.2 μM) and mefloquine (39.2±6.1 μM). Scatchard plots for the strong complexes also indicate that more than one acceptor molecule conformation may be involved in the PPIX complex formation especially at the high concentration range, leading to formation of more than one intermolecular structure for the PPIX-acceptor complexes.

Conclusions: In addition to aromatic ring systems, basic nitrogen centres are important for strong binding affinity. Molecules structurally related to chloroquine that showed strong PPIX complex association may also find use in the treatment of Porphyria. Animal toxicity studies will not normally be required for the already existing drugs.

Drug Delivery & Pharmaceutical Technology

118. Mechanistic Evaluation of Cholesterol Absorption Inhibitors using *in vitro* Lipolysis Model: Nanoscale Aluminosilicate and Cholestyramine

Pavel Gershkovich[1,*], Olena Sivak[1], Susana Contreras-Whitney[1], Jerald W. Darlington[2], and Kishor M. Wasan[1]. [1]Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada; [2]AMCOL International Inc

Purpose: To assess the effect of a novel cholesterol absorption inhibitor protonated nanoscale aluminosilicate (NSAS) and cholestyramine on intestinal processing of cholesterol

Methods: Both NSAS and cholestyramine were evaluated at three doses (262.5mg, 175mg and 87.5mg) using in vitro dynamic lipolysis model comprised of a pH-stat titration unit and continuously stirred and temperature-controlled lipolysis medium (containing digestion buffer at pH 7.4, bile salts, phospholipids, peanut oil, cold cholesterol and radiolabeled [3H]Cholesterol). The lipolysis process was initiated by the addition of 1000 IU/ml of pancreatin extract. Following the completion of the lipolysis the medium was ultracentifuged and the distribution of cholesterol between lipid, micellar (aqueous) and sediment phases was determined by means of radioactivity.

Results: In control experiments without cholesterol absorption inhibitors the majority (86.8 ± 4.6%) of cholesterol was recovered in micellar phase. NSAS redistributed cholesterol from micellar to sediment phases in a dose dependent manner with 85.9 ± 7.8% of cholesterol in the sediment at the highest dose of NSAS (262.5mg). Cholestyramine redistributed cholesterol into both sediment and lipid phases in a dose dependent manner with 20.5 ± 4.2% and 50.7 ± 8.7% of cholesterol in sediment and lipid phases, respectively, at 262.5mg dose of cholestyramine. The absence of bile acids in the system (conditions that mimic the maximal effect of cholestyramine) resulted in lower distribution into sediment phase than in presence of cholestyramine (2.3 ± 0.3% and 44.9 ± 12.1% in the sediment and lipid phases, respectively).

Conclusions: NSAS induced sharp redistribution of cholesterol from aqueous to sediment phase which

suggests that NSAS exerts its inhibition of cholesterol absorption by direct or indirect binding to cholesterol, its precipitation and excretion with feces. Cholestyramine induced redistribution of cholesterol into both oil and sediment phase which suggests potential cholesterol-lowering activity of this compound on the level of intraluminal processing of cholesterol, in addition to generally accepted theory of increase in endogenous bile acids synthesis. Assessment of intestinal processing of cholesterol using *in vitro* dynamic lipolysis model can potentially become a valuable approach to assess the action of cholesterol absorption inhibitors in fast and efficient manner.

*Part of this work was presented at AAPS 2011 Annual Meeting and Exposition.

119. Gelucire® 50/13 for Enhancing the Dissolution Rate and Intestinal Permeation of Gliclazide

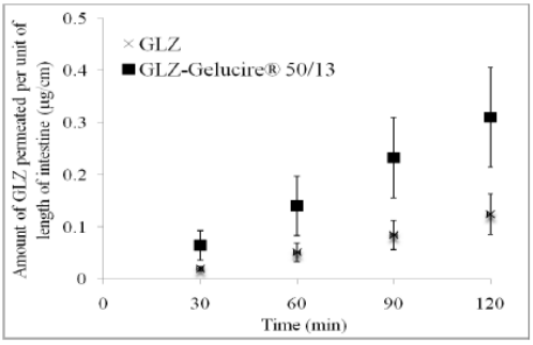
Abdullah H. Alomrani, Pharmaceutical Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Purpose: Gliclazide (GLZ) is a hypoglycaemic agent belongs to group of sulfonylurea. GLZ experiences inter-individual variation in bioavailability which is manifested by non-predictable t_{max}. The slow dissolution rate and incomplete intestinal absorption, due to p-glycoprotein (p-gp) efflux, of GLZ were proposed as a possible reason for such variation in bioavailability. The majority of the published studies are based on resolving problem associated with dissolution rate of GLZ utilizing different techniques (such as solid dispersion, complexation, micellization… etc), and ignored the possibility of efflux effect. The current study investigated the impact of binary solid dispersion system of GLZ-Gelucire® 50/13 on the dissolution rate and intestinal permeability of GLZ.

Methods: GLZ, in the presence of different concentration of Gelucire® 50/13, was dissolved in dichloromethane and spray dried using Buchi Mini Spray Dryer B-290. The yielded spray dried forms of GLZ- Gelucire® 50/13 binary systems were characterized with respect to their thermal behaviour and drug dissolution as well as intestinal permeability of GLZ in the presence and absence of Gelucire® 50/13.

Results: The results of the thermal analysis of GLZ revealed a significant reduction in the transition temperature (Tm) of spray dried form of GLZ in the presence of Gelucire® 50/13 compared to unprocessed one. The dissolution data indicated the ability of Gelucire® 50/13 to enhance GLZ dissolution rate in an acidic medium. GLZ-Gelucire® 50/13 binary system in weight ratio of (1:0.3) showed release of more than 75% of GLZ within the first 10 min of dissolution time compared to 8% in case of unprocessed GLZ. The intestinal permeability of GLZ in the presence of Gelucire® 50/13 was significantly enhanced by more than 50% compared to control (GLZ alone).

Parameter	GLZ unprocessed	GLZ-Gelucire® 50/13 (1:0.3)
Tm (°C)	172.1 (0.4)	163.2 (0.6)
% of GLZ released in 10 min	8.2 (1.4)	80.7 (7.6)

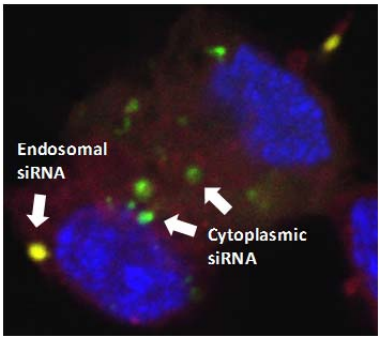


Conclusion: Binary solid dispersion system of GLZ with Gelucire® 50/13 was effective to enhance dissolution rate of GLZ as well as its intestinal permeability. The enhancement of intestinal permeability could be attributed to the ability of Gelucire® 50/13 to inhibit intestinal P-gp efflux.

120. Potentiated Silencing Following the Co-delivery of Anti-VEGF and Anti-STAT3 siRNA to Human Colorectal Carcinoma Cells (HCT 116) Using Lysine-Functionalized G□C Rosette Nanotubes

Aws Alshamsan[1,2], Uyen Ho[2,3], Mounir El Bakkari[2,3], and Hicham Fenniri[2,3]. [1]Nanomedicine Research Unit, 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: Effective delivery remains the main obstacle against therapeutic potentials of the small-interfering RNA (siRNA). We have previously shown complete association of siRNA with the supramolecular structures of twin guanine-cytosine motifs (G∧C) decorated with oligolysine moieties (Kn.T) i.e. lysine-functionalized rosette nanotubes (K-RNTs). We have documented that siRNA serves as an initiator of the self-assembly process, where siRNA molecules may interact with surface lysine residues as well as get entrapped into the K-RNT. In this study, we address whether these K-RNTs could serve as effective delivery systems to mediate siRNA silencing in cancer cells.
Methods: In sterile tubes, increasing mole ratios (0.5-20) of $Kn_{(1-15)}$.T were incubated with 1 mole of siRNA for 45 minutes in 37°C in sterile PBS (pH 7.2). Gel retardation assay has been employed to determine siRNA binding. The protective effect of K-RNTs on siRNA has been studied in presence of FBS. siRNA uptake by HCT 116 has been screened and evaluated for all $Kn_{(1-15)}$.T. Thereafter, timely-fashioned cell uptake was studied over 96 h for siRNA complexed with K5.T, K10.T, and K15.T using fluorescence microscopy. Moreover, confocal laser scanning microscopy has been used to evaluate the compartmentalization and endosomal escape of siRNA/K-RNT following cell uptake. Non-specific toxicity of these composites on colorectal carcinoma was assessed by MTT assay. To assess siRNA silencing ability, ELISA and western blot were used to detect VEGF and activated STAT3 (p-STAT3), respectively.
Results: Gel electrophoresis studies showed complete binding of siRNA with 20 moles of $Kn_{(4-15)}$.T. Similarly, protection of siRNA from serum degradation has been seen within the same range. Remarkable cell uptake of siRNA started with K4.T and increased as a function of the lysine chain length. Fluorescent siRNA signal increased with time especially between 24 h to 36 h and could be still detected after 96 h of incubation. Confocal images showed co-localization of siRNA and endosomal dyes along with free siRNA signals in the cytoplasm. Using 100 nM of anti-VEGF and anf-STAT3 siRNA, VEGF concentration and p-STAT3 levels were remarkable reduced following co-delivery in K10-RNTs as compared to delivery of each siRNA alone indicating possible synthetic lethality. Nonetheless, it is still early to draw such a conclusion without detailed cytotoxicity studies.



Conclusion: K-RNTs can effectively deliver functional siRNAs to human coloreactal carcinoma cell line HCT 116, where co-delivery of two anti-cancer siRNAs might be of more therapeutic value.

121. A Short DNA Aptamer that Recognized TNF-alpha and Blocks its Activity *in vitro*

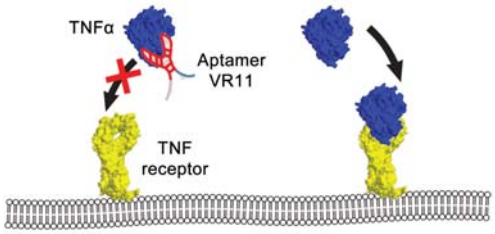
Erik W. Orava[a,c], Nick Jarvik[d], Sachdev S. Sidhu[d], Jean Gariépy[a,b,c,*]. [a]Department of Pharmaceutical Sciences, University of Toronto, Toronto, ON; [b]Department of Medical Biophysics, University of Toronto, Toronto, ON; [c]Sunnybrook Research Institute, Toronto, ON, [d]Terrence Donnelly Center for Cellular and Biomolecular Research, and Banting and Best Department of Medical Research, Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

Purpose: Unregulated immune responses are intimately associated with degenerative diseases such as atherosclerosis, arthritis, encephalitis, and tumours. Tumour necrosis factor-alpha (TNFα) is a pivotal component of the cytokine network linked to inflammatory diseases. Protein-based, TNFα inhibitors have proven to be clinically valuable.
Methods: In the present study, we constructed a 25-

nucleotide variable region DNA library to perform SELEX [Systematic Evolution of Ligands by Exponential enrichment] screens using recombinant TNFα as our target.

Results: We report the identification of short, single stranded DNA aptamers that bind specifically to TNFα. One such 25-base long aptamer, termed VR11, was shown to inhibit TNFα signalling as measured using NF-κB luciferase reporter assays. This aptamer bound to TNFα with a dissociation constant of 7.0±2.1nM as measured by surface plasmon resonance and specificity of this aptamer was confirmed using EMSA. Aptamer VR11 was also able to prevent TNFα-induced apoptosis as well as to reduce nitric oxide (NO) production in cultured cells for up to 24 hours. VR11 has a predicted G-quadruplex structure which may explain its stability in culture medium.

Conclusion: These studies suggest that VR11 may represent a simpler, synthetic scaffold than antibodies or protein domains upon which to derive oligonucleotide-based inhibitors of TNFα.



122. pH-responsive Lipids Based on a Molecular Switch for the Improved Delivery of Nucleic Acids

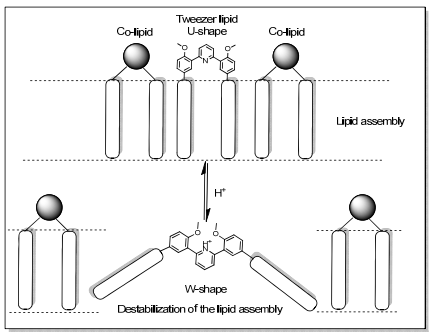
Jeanne Leblond Chain. Faculty of Pharmacy, University of Montréal, Montreal, QC, Canada

Purpose: Acid-responsive liposomes enable targeted release in cellular organelles or in tumoral tissues. In this work, a pH-responsive lipid has been designed using a molecular switch. At neutral pH, the U-shape conformation imposed by the tweezer unit promotes the formation of bilayer structures (see figure). The conformational switch to a W-shape would destabilize hydrophobic interactions and create defects in the bilayer, which have been shown to increase the endosomal escape ability.

Methods: Two approaches have been investigated for the synthesis of the lipid. Divergent method consisted in the Suzuki coupling of 5-bromo-2-methoxyphenyl boronic acid with 2,6-dibromopyridine. Resulting 2,6-bis(5-bromo-2-methoxyphenyl)pyridine was engaged in Suzuki or Kumada-Corriu-Tamao coupling with aliphatic chains. Convergent method started with Sonogashira coupling of 4-iodoanisole and 1-tetradecyne, which was reduced by catalytic hydrogenation. Subsequent bromination and reaction with bis(pinacolato)diboron in the presence of KOAc and $PdCl_2$dppf catalyst led to the corresponding boronic ester. The latter was engaged with 2,6-dibromopyridine in Suzuki coupling conditions, using $Pd(PPh_3)_4$ and Na_2CO_3. The reaction was completed in 4h using microwave irradiation (90°C, 1bar).

Results: The divergent method, in two steps, was the most straightforward. However, the first Suzuki coupling led to multiple side-products due to the presence of bromine on the boronic acid. Careful optimization of the parameters afforded the tweezer central unit in 67% yield. Nevertheless, neither the second Suzuki coupling nor Kumada-Corriu-Tamao coupling afforded the tweezer lipid, probably due to the low reactivity of the aliphatic chain. Alternatively, the convergent route consisted in assembling first the aliphatic arms of the tweezer, bridging them ultimately with the pyridine unit. This pathway offers the advantage of good-yielding reactions, such as Sonogashira coupling (80%), catalytic hydrogenation (89%) and bromination (78%). Moreover, the final Suzuki coupling was conducted upon microwave irradiation, which enabled the completion of the reaction in 4h instead of several days in classical conditions. Although longer than the divergent route, this latter synthetic route afforded the tweezer lipid successfully.

Conclusion: A lipid based on a molecular switch has been designed and synthesized. Its formulation with DOTAP into liposomes and its ability to trigger the release of an encapsulated dye (calcein) are currently investigated.



123. Doxorubicin-Mitomycin C Loaded Polymer Lipid Nanoparticle (PLN) Outperforms Doxil© with Increased Efficacy and Low Toxicity against Human Breast Tumor Model

Preethy Prasad[1], Ping Cai[1], Adam Shuhendler[1], Andrew M Rauth[2], and Xiao Yu Wu[1]. [1]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario; [2]Ontario Cancer Institute, Princess Margaret Hospital, University Health Network, Toronto, Ontario, Canada.

Purpose: Multidrug resistance (MDR) acquired by cancer cells and the dose-limiting toxicity of anti-cancer drugs are contributing factors to the failure of cancer chemotherapy. Doxorubicin (Dox) – Mitomycin C (MMC) co-loaded stealth polymer lipid hybrid nanoparticles (DMsPLN) were developed to overcome MDR and demonstrated anti-cancer synergy *in vitro*. The purpose of the study is to evaluate *in vivo* efficacy and safety of DMsPLN in a human breast tumor model.
Method: The efficacy and systemic toxicity of DMsPLN were evaluated against clinically used Doxil in nude mice bearing human mammary carcinomas. DMsPLN were administered intravenously at 50 mg/m^2 doxorubicin single dose or once every 4 days for 4 cycles. Tumor size was measured as a function of time to determine therapeutic efficacy of the treatment and systemic toxicity was monitored by repeated measurement of body weight.
Results: In the treatment of MDA MB 435/LCC6/WT and MDR1 tumors, there was a significant difference in the efficacy of DMsPLN relative to Doxil. A significant tumor growth delay was evident with either single or 4 times doses of DMs PLN. In single dose and 4x dose DMsPLN groups, the TGD were significantly improved to 100% and 131% in WT, and 118% and 122% in MDR1 models. Nevertheless, the mean tumor growth delay (TGD) in the Doxil-treated mice was 22.3% and 30.9% respectively. Complete tumor disappearance was observed in 10% of DMsPLN and 30% in DMsPLN 4 x groups in the WT tumor model. In the MDR1 tumor model, 11% of the mice showed complete tumor disappearance in both DMsPLN and in DMsPLN 4x group. None of the saline control or DMsPLN group members showed weight loss nearing 20%; whereas severe toxicity was observed in mice treated with Doxil (50 mg/m^2) with losses of 20% of total initial body weight.
Conclusion: DMsPLN demonstrated enhanced efficacy and reduced toxicity over Doxil in aggressive mouse models of sensitive and resistant human breast cancers. Therefore, DMsPLN may provide clinically relevant, more aggressive anti-cancer interventions.

124. Novel Cyclodextrin-based Nanoparticles Engineered for the Treatment of Skin Cancers

Heather Duerksen[1], Deborah Michel[1], Masoomeh Poorghorban[1], Umashankar Das[1], Jonathan Dimmock[1], Jackson Chitanda[2], Ronald Verrall[2], Ildiko Badea[1]. [1]Drug Design and Discovery Research Group, College of Pharmacy and Nutrition, University of Saskatchewan; [2]Department of Chemistry, University of Saskatchewan.

Hypothesis: Rationally designed lipid-based nanoparticles will deliver cytotoxic agents into the skin and selectively kill cancerous cells.
Introduction: The most common types of skin cancer that are prevalent in the human population include melanoma, squamous cell carcinoma, and basal cell carcinoma. Current topical treatment options for skin cancers are limited, and thus there is a need for the development of new therapeutic approaches. In this research, novel nanoparticles were developed that efficiently destroy cancerous cells with minimal effect on normal tissues.
Materials and Methods: A novel cytotoxic compound NC 2067 was selected from the 1,5-diaryl-3-oxo-1,4-pentadienyl family. A new delivery system was designed based on cyclodextrin conjugated with a cationic gemini lipid (CDgem). The cytotoxic effects of NC 2067 in DMSO and in CDgem nanoparticles were evaluated in melanoma cells, basal cell carcinoma cells, and normal skin fibroblasts. The cytotoxic effects of the complexes were compared to the currently used chemotherapeutic agent melphalan.
Results: It was found that NC 2067 incorporated in the CDgem delivery system exhibited significantly higher cytotoxicity (IC_{50} of 0.86μM) compared to melphalan (IC_{50} of 82.2 μM) in melanoma cells. The NC 2067 in DMSO showed higher cytotoxicity (IC_{50} 0.466 μM) than the CDgem formulation due to the intrinsic toxicity of DMSO in the melanoma cells. In addition, the candidate anticancer agent NC 2067 in CDgem formulation showed the highest cytotoxicity towards the melanoma cell line, compared to two different basal cell carcinoma cell lines with IC_{50}

values of 24.8 µM and 12.5 µM, respectively. The nanoparticles showed no toxicity towards healthy keratinocytes and fibroblasts.

Conclusion: This novel nanoparticle-based delivery system has the potential to be especially beneficial in the treatment of melanoma, which lacks adequate treatment to date.

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

125. Nanonized Nystatin: From *In Vitro* Activity to *In Vivo* Efficacy Evaluation

Alexandre Melkoumov[1], Fatiha Louhichi[2], Arwa El-Housseini[1], Martine Raymond[2], Louis de Repentigny[3] and Grégoire Leclair[1]. [1]Faculty of Pharmacy, [2]Institute for Research in Immunology and Cancer and [3]Department of Microbiology and Immunology, Faculty of Medicine, Université de Montréal, Montreal, Canada.

Purpose: Nystatin is a molecule of choice for the treatment of oral candidiasis. This drug is almost insoluble in physiological fluids and is impermeable to the intestinal membrane. Reducing the particle size of nystatin increases its specific surface area, its surface of contact with the infectious agent and possibly its diffusion within the infected tissue. As previously reported by our group, nanonizing nystatin improved its activity *in vitro.* This study compared the *in vivo* efficacy of nanonized vs. regular suspensions of nystatin.

Methods: Regular suspension of nystatin is commercially available (PMS-Nystatin). A nanosuspension of nystatin of identical composition was prepared by milling the regular suspension using a high energy DynoMill ML (GlenMills). Nystatin concentration was determined by HPLC (Shimadzu Prominence) and particle size by laser diffraction (Coulter LS13320). *In vitro* activity against *Candida albicans* was evaluated on a solid medium using a yeast growth inhibition method (12.5 to 5000 µg/mL, 8 concentrations, n=9). *In vivo* efficacy was evaluated in immunosuppressed DBA/2 male mice (cortisone acetate s.c., 250 mg/kg, day -1, +1 and +3) according to a protocol approved by our animal care committee. The oral cavity of each mouse was inoculated with *C. albicans* on day 0. Animals were divided in 3 groups with n=10 per group: (A) nanonized nystatin, 40,000 IU/kg in 50 µL PBS (B) regular nystatin 40,000 IU/kg in 50 µL PBS and (C) 50 µL PBS. Daily treatments started one day after inoculation (day 1) and were continued for 13 days. *C. albicans* was sampled daily by rubbing the oral surface of each animal with a Calgiswab. The Calgiswab used for sampling was dissolved in Ringer's solution and plated on Sabouraud dextrose agar. The plate was incubated for 24 h at 37°C, and the total colony forming units were measured.

Results: A nystatin nanosuspension (x_{50} < 0.150 µm) was successfully prepared from a regular suspension (x_{50} = 6.6 µm). HPLC results were similar for both formulations. *In vitro* activity is reported in the following table (100 to 5000 µg/mL, as some lower concentrations showed non-detectable diameters). *In vivo* experiments are ongoing and these results will be presented at the meeting.

Concentration (µg/mL)	Nanonized Nystatin (cm)	Regular Nystatin (cm)	p
100	1.01 ± 0.15	0.85 ± 0.11	0.02
250	1.18 ± 0.06	0.93 ± 0.13	0.0003
500	1.24 ± 0.09	0.99 ± 0.13	0.0003
1000	1.32 ± 0.08	1.07 ± 0.19	0.004
5000	1.44 ± 0.10	1.20 ± 0.14	0.001

Conclusion: A nystatin nanosuspension was successfully prepared from a regular suspension while maintaining its composition and assay. This nanosuspension demonstrated improved activity *in vitro*. Results from the *in vivo* study will be used to evaluate the need for further development and eventually a clinical efficacy study.

Acknowledgement: Arwa El-Housseini is an awardee or the National Summer Student Research Program Awards to Present Research Findings sponsored by GlaxoSmithKline Inc.

126. Effect of Material Hydrophobicity on the Permeability of Insulin through Porous Membranes

Jason Li, Michael Chu, Azhar Z. Abbasi, Claudia Gordijo and Shirley Wu. Faculty of Pharmaceutical Sciences, University of Toronto, Toronto, Canada

Purpose: Porous membranes are an important component for many implantable insulin delivery systems as they serve as permeable or semi-permeable interfaces between a source of insulin and

the host tissue (e.g., encapsulated islet cells, membrane-reservoir systems). While porous membranes may be manufactured from a number of biocompatible materials, insulin permeability through the membranes will depend largely on the surface interaction between insulin and the membrane material. Here we examine the effect of material hydrophobicity on the permeability of insulin through a porous membrane.

Methods: Porous membranes with precisely controlled dimensions (micron-sized pore diameters and membrane thickness) were manufactured from two non-degradable biocompatible polymers: ethylene-vinyl acetate (EVAC) and polydimethylsiloxane (PDMS). PDMS membranes were further treated with oxygen plasma or polyethylene glycol (PEG) of different molecular weights (2kDa or 20kDa) to alter their surface hydrophobicity. Successful conjugation of PEG molecules onto membrane surfaces was verified using XPS. Uniform membrane dimensions were confirmed using SEM. The hydrophobicity of each membrane was determined using contact angle measurements. Membranes were placed between an insulin reservoir containing 100 mg/ml of insulin in a PBS buffer (pH 7.4) and a receiver cell filled with PBS. Insulin permeability through the membranes was measured using UV absorbance at 276 nm over a 4 hour period. All experiments were conducted under constant mixing conditions and maintained at 37^{o}C (n=4 for each membrane). Circular dichroism was used to confirm structural integrity of the permeated insulin.

Results: Contact angle measurements revealed different degrees of hydrophobicity amongst the various membranes, with EVAC membranes being the most hydrophobic and 20kDA PEG PDMS membranes being the most hydrophilic. Compared to EVAC membranes, increasing insulin permeation was measured for untreated, oxygen plasma, 2kDA PEG, and 20kDA PEG-treated PDMS membranes.

Conclusion: Material hydrophobicity was found to be a key parameter in determining insulin permeation through a porous membrane. Insulin permeability is significantly elevated through hydrophilic membranes (PDMS treated with oxygen plasma or PEG) as compared to those made from EVAC and untreated PDMS. The degree of hydrophilicity further correlated with the rate of insulin permeation, as evidenced by the faster insulin permeation through membranes with lower measured contact angles. These results suggest that the presence of hydrophobic surfaces cause nonspecific adsorption of insulin onto the membrane, thus decreasing insulin permeability. Therefore, insulin delivery through a porous or semi-permeable membrane is maximized when hydrophilic materials or surface treatments are employed for membrane construction.

127. Enhanced Anticancer Activity by Doxorubicin-Mitomycin C Co-Loaded Polymer Lipid Nanoparticles in Triple Negative and Herceptin®-Resistant Human Breast Cancer Cells

Dan Shan[1], Preethy Prasad[1], Andrew Mike Rauth[2], Xiao Yu Wu[1]. [1]Leslie Dan Faculty of Pharmacy, University of Toronto, [2]Ontario Cancer Institute, Toronto, Canada

Purpose: Doxorubicin (DOX) and mitomycin C (MMC) co-loaded polymer lipid nanoparticle (DMsPLN) system was demonstrated, in our previous studies, to generate synergistic anticancer activities and to overcome multidrug resistance (MDR) in breast cancer cells that overexpress P-gp, MRP1 and BCRP efflux pumps. The aim of current study was to investigate the anticancer activity of DOX-MMC combination in solution form and their co-delivery by the PLN system against triple negative and Herceptin®-resistant breast cancer cells.

Methods: Polymer-lipid hybrid solid nanoparticles were synthesized with a fatty acid lipid core and a poly(ethylene glycol) corona. MMC was directly loaded into the nanoparticle while DOX was complexed with hydrolyzed polymer of epoxidized soybean oil for optimal drug loading. *In-vitro* anticancer efficacy of free DOX or MMC, free DOX-MMC combination, DOX-loaded PLN (DOXsPLN), MMC-loaded PLN (MMCsPLN) and DMsPLN were examined by incubating the formulations at various doses with triple negative MDA-MB-468 or Herceptin® resistant MDA-MB-231 human breast cancer cell lines for 24 hours. The amount of cell kill was assayed by MTT assay. The median effect dose and the combination index values of DOX with MMC were determined using the median effect analysis.

Results: The combination therapy of DOX and MMC with a molar ratio of 1:1.4 significantly enhanced the cell kill of both MDA-MB-231 and MDA-MB-468 cells as compared to individual drug treatments. The combination index values were used to analyze whether the cytotoxicity was additive or

synergistic. The values indicated that, at lower dose (e.g. 0.5μM DOX), the combination treatment was additive for both free drug and nanoparticle formulation. At higher doses, the combination index dropped to values smaller than unity, suggesting a synergistic effect of the DOX-MMC combination. The nanoparticle formulations, i.e., DOXsPLN, MMCsPLN and DMsPLN were more effective in cell kill than the free drugs in both cell lines indicating increased efficacy using PLN delivery system.

Conclusion: The combination therapy of DOX and MMC has shown a synergistic effect and co-encapsulation of the drugs in PLN enhanced anticancer activity in both triple-negative and Herceptin®-resistant human breast cancer cells.

128. Reduction of Cardiotoxic Metabolite of Doxorubicin in Blood and Heart of Breast-Tumour Bearing Mice by Encapsulation Doxorubicin in Polymer Lipid Hybrid Nanoparticles

RuiXue Zhang[1], King Chen[1], Ping Cai[1], Andrew M. Rauth[2], and Xiao Yu Wu[1]. [1]Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto; [2]Ontario Cancer Institute, University Health Network, Toronto, Canada

Purpose: The clinical usage of a well-known anticancer drug, doxorubicin (DOX), is limited by its accumulative cardiotoxicity, which is commonly associated with the formation of the secondary alcohol metabolite of DOX, doxorubicinol. Liposomal doxorubicin, i.e. DOXil® (Caelyx), can reduce the DOX-induced cardiotoxicity, but DOXil® does not show any improved efficacy compared to free DOX. Our laboratory has developed an innovative polymer-lipid delivery system (PLN) that is capable of simultaneously encapsulating both DOX and mitomycin C (MMC). This formulation has demonstrated improved efficacy and reduced toxicity in breast-tumour bearing mice *in vivo* comparing to free DOX and DOXil®. Yet, the formation of doxorubicinol, has not been evaluated for dual anticancer drugs loaded PLN. To better understand mechanism underlying lower toxicity but higher efficacy associated with this PLN system, we assessed the formation of the metabolite, doxorubicinol, in the blood and the heart of breast-tumour bearing mice treated by PLN encapsulating DOX with/without MMC as compared to free drugs.

Methods: Orthotopic murine breast tumors were induced by injecting 3×10^6 EMT6 cells in the right mammary fat pat of 8 to 10 weeks-old Balb/c mice. After the larger diameter of tumour reached 5mm, a single dose of free DOX (10 mg/kg), free DOX with MMC, DOX-loaded PLN (DOX-PLN), and DOX-MMC co-loaded PLN (DMPLN) was injected to the mice intravenously. The blood samples and the heart tissues were collected at various time points and the concentration of doxorubicinol was analyzed by LC-MS/MS.

Results: The mice treated with PLN encapsulated DOX showed at least 2-fold lower concentration of doxorubicinol in the blood compared to the groups treated with free DOX, DOXil®, or free DOX-MMC combination. Moreover, the mice treated with DOX-PLN and DOXil® had 3 to 9-times lower accumulation of doxorubicinol in the heart than free DOX.

Conclusion: The formation of the cardiotoxic metabolite doxorubicinol in the blood and the heart was significantly lowered with the PLN formulation. The results suggest that the PLN formulation is possible to reduce myocardiotoxicity and myoelosuppression caused by DOX combinational therapy.

129. Synthesis of PEG Grafted Polyester Polymers to Improve Targeted Delivery of Nanocarriers

Jean-Michel Rabanel[1] & Patrice Hildgen[1]. [1]Faculté de Pharmacie, Université de Montréal, Montréal, QC, Canada

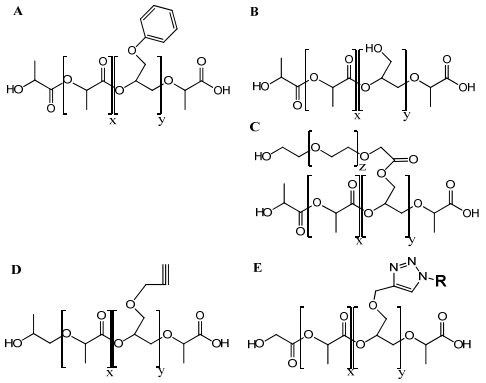
Purpose: Poly(ethylene glycol) grafted on poly(D,L lactide) (PEG-g-PLA) polymers have proved their usefulness in the preparation of nanosized targeted drug delivery systems. However, the ability of these nanocarriers to selectively target theirs site of action in vivo is limited (usually no more than 10% of the injected dose). In order to improve the targeting efficiency, one approach is the optimisation of their surface architecture. In particular, this objective can be fulfilled by a precise control of PEG content on their surface and the presence of functional groups in the polymeric chain allowing specific ligands to be covalently coupled. In this work, we propose to produce copolymers of lactic acid with benzyl glycidyl ether (BGE) and/or propargyl glycidyl ether

(PGE). The added pendant groups will allow grafting on the polymer backbone of a controlled quantity of PEG, fluorescent dyes and model ligands suitable for preparation of targeted drug nanocarriers.

Method: Random copolymerization of D,L dilactide in presence of variable molar ratio of BGE (BGE/lactic acid ratio) and/or PGE were carried out by ring-opening polymerization catalyzed by stannous 2-ethyl hexanoate (molar ratio of 1/5000) at 150°C under argon atmosphere. After polymer purification by precipitation, alcohol pendant group were uncovered by catalytic hydrogenation in presence of Pd/Carbon. Methoxy PEGs (2kD) were grafted by acylation to yield Peg-g-PLA. Rhodamine comprising an azide group was grafted on the terminal alkyne group by click chemistry. The molecular weights of the polymers were characterized by GPC in THF with PS standards, ^{1}H NMR, ^{13}C NMR and 2-D ^{1}H/^{1}H (400 MHz) experiments were performed, as well as ATR-IR and DSC. LC/MS analysis of polymer fragments after complete hydrolysis in dilute NaOH solution was also performed.

Results: The structures of the resulting polymers are shown in the figure below: (A) Copolymer of BGE with dilactide; (B); (A) after catalytic hydrogenation (OH pendant group) (C) PEG-g-PLA; (D) Copolymer of PGE with dilactide; (E) (D) after click chemistry and grafting of a model ligand or fluorophore (R). Different analyses confirmed the structure of the polymers. BGE and PGE insertion ratio were found close to the feed ratio in the range tested (0.5-2%).

Conclusion: This study confirmed the feasibility of the synthesis of multifunctional polymers which could prove to be useful for the optimal design of targeted polymeric nanodevices. Moreover, we showed the complete degradation of the polymers failed to generate toxic material.



130. Distribution of Effervescent Inhalable Nanoparticles after Pulmonary Delivery; In vivo Study

M. Al-Hallak,[1,2] M. Sarfraz,[1] S. Azarmi,[1,4] W. Roa,[5] W. Finlay,[6] C. Rouleau[7], and R. Löbenberg[1*].
[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada; [2]Faculty of Pharmacy, Damascus University, Damascus, Syrian Arab Republic; [4]Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran; [5]Cross Cancer Institute, University of Alberta, Edmonton, AB; [6]Department of Mechanical Engineering, University of Alberta, Edmonton, AB; [7]Fisheries and Oceans Canada, Maurice-Lamontagne Institute, Mont-Joli, Canada.

Purpose: Pulmonary delivered nanoparticles is promising alternative route for conventional lung cancer treatment in animals. The purpose of study is to investigate the bio-distribution of effervescent inhalable Nanoparticles (NPs) after single dose in lung cancer bearing mice and determine the efficacy of this route and dosage form in lung cancer treatment.

Methods: Whole-body autoradiography (WBA) and confocal laser scanning microscopy (CLSM) were used to investigate the distribution of inhalable NPs loaded in an effervescent micro-carrier. Inhalable NPs were tagged with ^{14}C for the WBA, or with fluorescein isothiocyanate (FITC) for (CLSM) imaging. The entire effervescent nanoparticles were characterized for size, purity and drug loading efficacy before used. The non-small cell lung carcinoma cell line NCl-H460 was used to induce cancer. Two weeks after inoculation with cancer cells, the mice were divided into three groups: each group received 1 mg of inhalable radioactive effervescent DOX-loaded nanoparticles, FITC-labeled effervescent inhalable DOX-loaded NPs and ^{14}C-labelled DOX solution. The inhalable NPs in groups 1 and 2 were administered via a pulmonary route and the DOX solution was administered via tail vain injection.

Results: The results showed that post-inhalation, NPs were widely disseminated in the lungs with long retention time (24 hours). The heart was radio-activity free at all the time points of the study. CLSM images showed that inhalable NPs were up-taken by cells and that doxorubicin was released to the cell nuclei. The ability of inhalable NPs to achieve deep lung deposition, to be actively released from micro-carrier particles, to spread to different

parts of the lung, and to release DOX in vivo all contribute to the efficacy of the effervescent inhalable NPs as lung cancer treatment.
Conclusion: Autoradiography showed that NPs were able to achieve deep lung deposition and disseminate to different parts of the lung and to some extra-pulmonary tissues. The long retention time (24 hours) of inhalable NPs in the lungs can explain the efficacy and safety of the inhalable NPs. CLSM images indicated that DOX reached cell nuclei to exert anticancer activity. The ability of inhalable NPs to achieve deep lung deposition, to be actively released from microcarrier particles, to spread to different parts of the lung, and to release DOX in vivo all contribute to the efficacy of the effervescent inhalable NPs in lung cancer treatment.

131. A 3-D Tissue Model for the Assessment of Nanoparticle Penetration in Tumor Tissues

Andrew Mikhail[1,2], Sina Eetezadi[1] and Christine Allen[1,2]. [1]Leslie Dan Faculty of Pharmacy and the [2]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Canada.

Purpose: Poor penetration and distribution of chemotherapeutic agents in solid tumors has been recognized as one of the major challenges limiting the efficacy of macromolecular and nanoparticle-based cancer therapies. While the augmented tumor accumulation of drugs encapsulated in nanoparticles has been recognized, corresponding improvements in therapeutic efficacy have often been incommensurate. Initial investigations into the intratumoral fate of macromolecules and nanoparticles have suggested that they are heterogeneously distributed and display limited penetration into avascular tumor compartments. In the present work, the tissue penetration and intratumoral distribution of BCMs were explored in 3-D multicellular tumor spheroids (MCTS) in an attempt to establish a high-throughput in vitro platform for evaluating the influence of nanoparticle properties on their interstitial transport. The penetration of micelles of difference sizes into MCTS formed by different cell lines was examined.
Methods: Fluorescently labeled micelles were prepared from PEG-*b*-PCL copolymers conjugated with a fluorescent probe by hydration of dried copolymer films. MCTS were generated using human cervix (HeLa) and colon (HT29) cancer cells seeded onto non-adherent 96-well round-bottomed plates. For evaluation of nanoparticle penetration, MCTS were incubated with 15 (BCM-15) and 55 (BCM-55) nm fluorescently labeled BCMs for 1 and 24 h. MCTS were washed, frozen and cryosectioned prior to imaging using a fluorescence microscope. The total fluorescence intensity per unit area was calculated with respect to depth from the MCTS surface using a custom designed MATLAB algorithm.
Results: MCTS possessed properties reminiscent of tumors including gradients in cellular proliferation and regions of necrosis. Algorithm-based image analysis revealed that after 1 h, BCM-15 demonstrated a homogeneous radial distribution across both HeLa and HT29 MCTS while distribution of BCM-55 remained higher at the MCTS periphery. However, after 24 h of incubation both BCM-15 and BCM-55 achieved a homogeneous radial distribution throughout the MCTS. Furthermore, the total signal intensity and thus the total penetration of BCMs in the MCTS remained dramatically higher at all radial position in HeLa MCTS when compared to HT29.
Conclusion: A robust procedure for growing MCTS was developed allowing for high-throughput analysis of nanoparticle diffusive transport in 3-D tumor tissues. Transport of BCMs in MCTS depended on the MCTS cell line, incubation time and size of the nanoparticles.

132. Encapsulation of Voriconazole into PEG-grafted-PLA Nanoparticles: Preparation, Characterization and Antifungal Activity

Valery Aoun[1], Patrice LePape[2], Patrice Hildgen[1] and Grégoire Leclair[1]. [1]Faculté de pharmacie, Université de Montréal, Montréal, Canada; [2]Département de Parasitologie et Mycologie Médicale, Université de Nantes, France

Purpose: We propose to improve the antifungal activity of voriconazole by loading this drug into PEG-*grafted*-PLA nanoparticles (NP/VRZ). NPs/VRZ were characterized for their physicochemical properties and antifungal activity was tested against *Candida spp* and *Aspergillus fumigatus*, two fungi pathogens target of conazole drugs. The novel formulation was also evaluated against *Candida spp* biofilms.
Methods: Methoxy-PEG (2000 Da) and Diol-PEG (2000 Da) were grafted on PLA backbones (5%

grafting density). NP/VRZ were prepared using an O/W solvent evaporation method. The encapsulation efficiency (HPLC), shape and morphology (AFM), surface charge (Malvern Zetasizer, zeta potential mode), size (Malvern Zetasizer, DLS mode) and thermal (DSC) analyzes were performed on the NP/VRZ. The antifungal activity was obtained by determination of the minimum inhibitory concentration at 50% (MIC50) using a growth inhibition method against *C. albicans* and *A. fumigatus* cultures. MIC50 was evaluated after 24 hours and 48 hours by spectrofluorometry (excitation and emission: 590 nm). A MIC50 specification of 1mg/mL was utilized to differentiate susceptible and non-susceptible cultures as defined by the Clinical and Laboratory Standards Institute. A similar assay was performed using *Candida spp* biofilms.

Results: Typical NP/VRZ had a mean size value of 150 nm by DLS. This size was confirmed by AFM which also revealed that NP/VRZ were spherical with a smooth surface. A drug loading efficiency of 1.3% (w/w) was measured. *In vitro* antifungal activity results of NP/VRZ and free VRZ against *C. albicans* and *A. fumigatus* strains are reported in Table 1. The MIC50 of NP/VRZ was around 9 times lower than the MIC50 of free VRZ against *Candida spp.*

Table 1. MIC50 (μg/ml) of VRZ and NPs/VRZ against *C. albicans (1)* and *A. fumigatus (2)* strains

Strains		Formulations	
		VRZ	NPs/VRZ
CAAL 67		0.471±0.161	0.053±0.005
DSY 735	**(1)**	0.354±0.031	0.053±0.006
ATCC 6258		0.348±0.042	0.039±0.009
70056323		0.086±0.014	0.179±0.019
70041901	**(2)**	0.394±0.014	0.723±0.006
AF 350		0.085±0.058	0.182±0.116

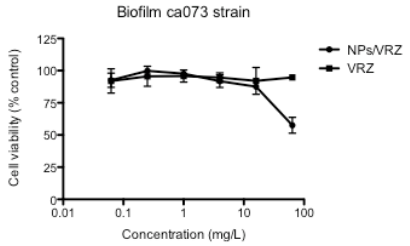


Figure 1. Cell viability curve on biofilm of ca073 strain

For *A. fumigatus* strains, the MIC50 was 2-fold lower for free VRZ than for NP/VRZ. The activity of the new formulation was tested on *C. albicans* biofilms using to various drug concentrations. The results reported on Figure 1 are representative of all 6 strains tested. As shown of this graph, the activity of NP/VRZ was slightly improved at the highest concentrations.

Conclusion: NP/VRZ were prepared successfully and provided an improved antifungal activity against *Candida spp* cells and biofilms. The potential applications of polymeric nanoparticles in the field of mycology offer several opportunities as this area of research has not yet been fully explored.

133. Improving the Pharmacokinetics, Drug Delivery, Antitumor Efficacy and Safety of Docetaxel via Preparation of a Carboxymethylcellulose Conjugate

Mark J. Ernsting, Elijus Undzys, Barry Press, Ahmed Aman, and Shyh-Dar Li. Drug Delivery and Formulation Group, Medicinal Chemistry Platform, Ontario Institute for Cancer Research, Toronto, Ontario, Canada

Purpose: Detergent formulations of taxane chemotherapeutics such as Taxol® and Taxotere® are approved for multiple cancer indications, but induce systemic side effects including neuropathy and neutropenia, conditions which limit the frequency and duration of treatments. Abraxane® is a nanoparticle formulation of paclitaxel, and while safer than Taxol, also induces serious side effects. We have synthesized a polymeric conjugate of docetaxel (DTX) around a PEGylated carboxymethyl cellulose backbone (Cellax): Cellax condenses into a nanoparticle and slowly releases DTX for 3 weeks.[1-3] In this study we compare the pharmacokinetics (PK), biodistribution (BD), efficacy and toxicity of Taxotere, Abraxane and Cellax.

Methods: 5 wt% polyethylene glycol (PEG) and 37 wt% DTX were coupled to an acetylated carboxymethylcellulose precursor, producing a polymeric construct (Cellax) that condenses into 110-120 nm particles.[1,2,3] The PK and BD of Taxotere, Abraxane and Cellax (40 mg taxane/kg) were studied in tumor bearing mice. Samples of blood and tissue were assayed for taxane by LC/MS at different time points post injection.[2] Antitumor efficacy and safety of these taxane formulations

were compared in an s.c. B16F10 tumor model.

Results: The PK parameters for Taxotere, Abraxane, and Cellax are summarized in Table 1: Cellax increased taxane $t_{1/2}$ by 11 fold compared to Taxotere and 20 fold compared to Abraxane, reduced plasma clearance and volume of distribution, and increased plasma AUC by ~30 fold. In biodistribution analysis, Cellax uptake was selective to the tumor with 5.4× and 203× increased accumulation compared to Taxotere and Abraxane, respectively. In the B16F10 model, Taxotere inhibited tumor growth by 60%, Abraxane by 96%, and Cellax by 99% at day 4. Tumor growth in the Abraxane group began rebounding after day 4, whereas Cellax controlled growth till day 12. Taxotere and Abraxane treated mice presented with severe neutropenia, an effect absent in Cellax treated mice.

Conclusion: Cellax significantly improved the PK profile of taxanes compared to Abraxane and Taxotere, increased the tumor delivery, enhanced efficacy and reduced toxicity in a mouse model.

References

1. Ernsting MJ et al. *Bioconjug Chem* 2011;22:2474-86.
2. Ernsting MJ et al. *Biomaterials* 2012;33:1445-54.
3. Ernsting MJ et al. *Biomaterials* 2012;33: 3931-3941.

Table 1: Pharmacokinetic parameters

	Taxotere	Abraxane	Cellax
t 1/2 (h)	11.1 ± 1.7	6.2 ± 1.2	125.2 ± 56.5
Mean residence time (h)	5.3 ± 2.7	1.8 ± 0.4	37.9 ± 11.9
Clearance (mL/h/kg)	1861 ± 401	1799 ± 435	34 ± 7
AUClast (h/ug/mL)	26 ± 4	22 ± 8	808 ± 127
Vz (mL/kg)	31614 ± 11835	16929 ± 5823	5088 ± 1116

134. High Concentration Pluronic-Insulin Gel Formulations with Improved Protein Stability and Bioactivity

Michael Chu, Claudia Gordijo, Chris Koo and Xiao Yu Wu. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Canada

Purpose: Insulin formulations have been designed with maintenance of stability and bioactivity in mind. As a protein pharmaceutical, insulin is subject to denaturation processes, such as shear forces, hydrophobic surface interactions and high temperatures. Storage of insulin for long periods requires resistances to these stresses, to maintain protein stability and in turn, bioactivity. As well, increasing concentrations of insulin can exacerbate these issues, making the maintenance of high concentration formulations difficult. This work was aimed to develop an insulin gel formulation that exhibits stability at physiological temperatures over a long term.

Methods: Pluronic-insulin gel formulations were prepared at a high concentration of 100mg/ml. Optimization of pH and zinc concentration was performed to determine best insulin stability. Samples were incubated at 37C up to a 30 day period to test long-term stability against thermal denaturation. Insulin stability was measured using circular dichroism (CD) and HPLC analysis to quantify resilience of protein structure. Incubated insulin was also diluted and injected into STZ-diabetic rat to determine its bioactivity at various incubation times.

Results: Pluronic-insulin gels created a highly viscous formulation that would not be subject to agitation or air-water interface denaturation stresses. Pluronic itself shows a hydrophobic-hydrophobic domain interaction, which prevents the surface interactions with proteins. As well, the addition of zinc provides a marked improvement on insulin stability as evidenced by CD spectra, showing a complete retention of secondary structure up to a 30 day incubation period. Bioactivity tests also showed complete maintenance of insulin activity after a 30 day incubation period with zinc. Insulin bioactivity lost significantly after 10 days without zinc.

Conclusions: A novel high-concentration insulin gel was prepared that is stable at physiological temperature, resistance to protein denaturation stresses seen in both physiological environments and shelf storage.

135. Using Peptides to Create Cancer Targeting Compounds

Christopher B. Chen, Rania Soudy, Kamaljit Kaur. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Breast cancer is the most likely cancer that an average Canadian woman can develop during her lifetime. Although there are several modalities to treat this type of cancer, there remain few

compounds that can effectively treat the cancer without inducing serious adverse effects in patients. In the past few decades, there has been an increase in the development of anti-neoplastics that effectively attenuate and eliminate breast cancer tumors while minimizing undesirable effects. To do this, many of the compounds use a targeting moiety that ensures that only breast cancer cells experience the cytoxicity of the compound. By minimizing the distribution of the compound to other cells, the targeting moiety diminishes the risk of adverse effects. Peptide 18-4 may be one of these moieties because it binds strongly to breast cancer cells and poorly to normal cells. We aim to conjugate peptide 18-4 to Doxorubicin (Dox), a compound capable of effectively eliminating cancerous tissue, to yield a peptide-drug conjugate that we can further test in selectivity and cytotoxicity assays with the hopes of developing a novel anti-neoplastic compound.

Methods: First, the amine group on Dox was protected by linking it to Fmoc-Osu to create (2). Next, glutaric anhydride was linked to the 14-OH on Dox to create (3) .This compound was then linked to peptide 18-4, which was synthesized using solid phase peptide synthesis, via glutaric anhydride to create our final product (4).

Results: We analyzed (2) and (3) with reverse phase high performance liquid chromatography. The spectrum for (2) resulted in a lone elution peak at 31 minutes and the spectrum for (3) resulted in peak at 39 minutes. Furthermore (2) and (3) were analyzed under matrix assisted laser desorption ionization, yielding readings of 788 g/mol and 902 g/mol respectively. By analyzing our suspected final product, (4), with the same mass spectrometry technique, we identified a lone peak at 1929 g/mol..

Conclusion: We have synthesized a compound with the potential to selectively and effectively treat breast cancer tumours. This allows us the progress to experiments where we will evaluate the biodistribution and cytotoxicty of this compound on neoplastic cells.

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

136. Preparation of Micelle-forming ABC Type Tri-Block Copolymers of Poly(ethylene glycol), Poly(lactide) and Poly(α-benazylcarboxylate-ε-caprolactone): The Effect of Stereo-chemistry on Micellar Properties in Drug Delivery

Hoda Soleymani Abyaneh, Mohammadreza Vakili, Afsaneh Lavasanifar. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Purpose: Polymeric micelles based on biodegradable block copolymers have been the subject of research for the solubilization of poorly water soluble drugs and development of depot as well as targeted drug delivery systems. A common problem with the polymeric micelles is the premature release of the incorporated drug from the carrier under sink condition observed following intravenous administration. The long term objective of this research is to design micellar structures that can enhance the solubility of hydrophobic drugs in their core and at the same time sustain the rate of drug release from the delivery system. In this study, we have synthesized ABC type tri-block copolymers composed of poly(ethylene glycol) (PEG), poly(DL lactide) (PDLA) or poly(L-lactide) (PLA), and poly(α-benzylcarboxylate-ε-caprolactone) (PBCL) and assessed the effect of polymer stereochemistry on relevant micellar properties in drug delivery.

Methods: ABC type tri-block copolymers of PEG-PDLA-PBCL and PEG-PLA-PBCL were synthesized using MePEG (5000 Da) as initiator, L-lactide (LA) or DL-lactide (DLA) as the monomer for the B block, and α-benzyl-carboxylate-ε-caprolactone as the monomer for the C block. Stepwise ring opening polymerization of monomers in the presence of stannous octoate was pursued for synthesis.

Results: Block copolymers of PEG-PLA-PBCL and PEG-PDL-PBCL formed micelles with average diameters of 63 and 61 nm, respectively. Transmission electron microscopy (TEM) revealed presence of spherical micelles for both polymers. The critical micellar concentrations (CMC), measured by fluorescence spectroscopy techniques, was 1.12 ± 0.08 and 1.41 ± 0.46 and μg/mL for PEG-PLA-PBCL and PEG-PDLA-PBCL, respectively. The ^{1}H-NMR peaks of PLA-PBCL or PDLA- PBCL were present in $CDCl_3$, but disappeared in D_20 after micellization, which

indicates the lactide-caprolactone core of the micelles were in a highly viscous state and chain mobility was restricted. Encapsulation of cucurbitacin I reached 100 % efficiency in both micellar structures. A slight but significant reduction in the release of cucurbitacin incorporated in PEO-PLA-PBCL micelles over PEO-PDLA-PBCL ones was observed in vitro release studies.

Conclusion: Synthesis of ABC block copolymers based on PEG-PLA-PBCL and PEG-PDLA-PBCL by ring sequential ring opening polymerization is a feasible approach. The results points to the influence of stereo-regularity in ABC block copolymer structure on micellar stability and drug release properties.

137. APA Microcapsules for the Delivery of *Lactobacillus fermentum* NCIMB 5221: An in-vitro Study

Catherine Tomaro-Duchesneau[1], Shyamali Saha[1,2], Mitchell L. Jones[3], Alain Labbé[3], Meenakshi Malhotra[1], Imen Kahouli [1,4], Laetitia Rodes[1], Satya Prakash[1*]. [1]Departments of Biomedical Engineering, Physiology, and Artificial Cells and Organs Research Center, Faculty of Medicine, McGill University, Montréal, Québec; [2]Faculty of Dentistry, McGill University, Montréal, Québec; [3]Micropharma Limited, Montréal, Québec; [4]Department of Experimental Medicine, McGill University, Montréal, Québec, Canada.

Purpose: Ferulic acid (FA), a hydroxycinnamic acid, is an antioxidant known to neutralize free radicals. These free radicals are involved in DNA damage, inflammation, cancer and aging. The administration of FA, as an oral therapeutic, is hampered by its absorption in the small intestine followed by its quick excretion. Colonic microbial enzymes have been shown to naturally produce FA. The delivery of a probiotic Lactobacillus that produces FA in the colon could, hence, prove beneficial for a number of health disorders. However, the delivery of a probiotic through the harsh conditions of the gastrointestinal tract (GIT) hampers the efficacy of any probiotic. A carrier system needs to be used to overcome the harsh conditions, while allowing successful probiotic enzymatic activity. In this research, alginate-polylysine-alginate (APA) microencapsulation was used to encapsulate *Lactobacillus fermentum* NCIMB 5221, a strain shown to produce FA in large quantities, in previous research by our group. The microencapsulated probiotic formulation was then characterized for its viability through a simulated GIT.

Methods: The alginate mix containing *L. fermentum* NCIMB 5221 at 8% (w/v) was passed through an Inotech microencapsulator with a 300 µm diameter nozzle. The bacterial-alginate droplets were gelated in a calcium chloride bath, followed by coating steps of polylysine and alginate. The microcapsules were incubated with ethyl ferulate (EFA) and FA production and EFA deconjugation were measured over time by high performance liquid chromatography. Survival in simulated gastric (SGF) and simulated intestinal (SIF) fluids was tested using colony forming units (CFU) on agar plates.

Results: Microcapsules of 400 ± 25 µm in diameter and viability of $1.21 \times 10^9 \pm 9.54 \times 10^7$ cfu/g were obtained. Our findings demonstrate that the APA microcapsule does not slow the mass transfer of substrate into and the FA product out of the microcapsule, while also not impairing bacterial viability during encapsulation. Exposure to SGF and SIF led to a significant 2.5 log difference in viability between free ($1.10 \times 10^4 \pm 1.00 \times 10^3$ cfu/mL) and microencapsulated ($5.50 \times 10^6 \pm 1.00 \times 10^5$ cfu/mL) *L. fermentum* NCIMB 5221 (table below).

Conclusion: The work presented here suggests that APA microencapsulation can be used as an effective oral delivery method for *L. fermentum* NCIMB 5221, a FA-producing probiotic. Future work using this strain should prove beneficial for the treatment/prevention of inflammatory diseases, cancer and aging.

Acknowledgement: The authors would like to acknowledge a Canadian Institute of Health Research (CIHR) grant (MOP 264308) to Dr. S. Prakash and a FRSQ Doctoral award to Meenakshi Malhotra.

138. Cytotoxicity Evaluation of the Novel Nanoparticle Formulations

Matthew Walliser, Sheikh Tasnim Jahan, Azita Haddadi. College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK.

Purpose: Poly (lactic-co-glycolic acid), or PLGA, nanoparticles are an intriguing novel drug delivery device. PLGA is both biocompatible and biodegradable, and has been FDA approved for use

as a drug delivery vehicle. It can be modified at the surface, allowing the nanoparticles to target specific endpoints in the body. This study will examine the cytotoxicity of the nano-conjugates.

Methods: We prepared the nanoparticles by the emulsification-solvent evaporation method, using PLGA of both ester and carboxyl-terminated end groups of different inherent viscosities (0.15-1.00 dL/g). Once prepared, nanoparticles were stored at -20 °C for future studies. The attachment of the ligand, a model antibody, was then performed by either covalent bonding to the carboxyl-terminated PLGA using the cross-linkers, or by physical adsorption to PLGA. The next phase in our studies was to conduct a toxicity testing of the nanoparticle-antibody conjugates in vitro. The cultures were treated with different nanoparticle formulations and control groups. Then, an MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) assay was performed to determine the cell viability.

Results: Nanoparticles were successfully prepared achieving an average nanoparticle size of 201.7±11.5 to 237.0± 22.2 for carboxyl-terminated PLGA nanoparticles depending on the polymer viscosity, while nanoparticles prepared by ester-terminated PLGA polymer showed an average size between 229.0±3.5 and 237.2±11.0. Surface charges for all the formulations were found to be in negative ranges. MTT assay showed that unmodified PLGA nanoparticles were not toxic to the cells and the conjugation did not increase the toxicity of the formulations.

Conclusion: PLGA nanoparticles are excellent delivery systems for various applications including cancer therapies, which is the purpose of our studies.

139. Effect of Single Protonable Nitrogen per Mole on Encapsulation of Plasmid DNA in Niosomal Core

Mohsen Bayomi[1,2], Abdullah Alomrani[1,2], Mounir Salem-Bekhit[1,2], and Aws Alshamsan[1,2,3]. [1]Nanomedicine Research Unit, Department of Pharmaceutics, College of Pharmacy, [2]Center of Excellence in Biotechnology Research, King Saud University, Riyadh, Saudi Arabia; [3]National Institute for Nanotechnology (NINT-NRC), University of Alberta, Canada.

Purpose: Nitrogen to phosphate (N/P) ratio represents the molar ratio of total protonable nitrogens on one mole of the cationic system to the total phosphates on the backbone of one mole of DNA. The importance of this ratio, as indicator of DNA entrapment efficiency, lies in the molar principle of this ratio. Unlike weight ratios, the N/P ratio provides more valid parameter as it corrects for the molecular weight of the delivery system unit, the number of positive charges, and the number of units used per system. However, high cationic density in any given delivery system raises the question of non-specific cellular toxicity. Here we investigate the capability of single protonable nitrogen per niosomal mole to encapsulate DNA plasmid at several N/P ratios.

Methods: Span 60 and cholesterol were mixed with a charge-inducing agent stearylamine (SA) or dioctadecyldimethylammonium bromide (DODAB) in presence of ethanol. The mixture was maintained at 60 ºC until complete solubility took place. Equal weight of MQ water was added with continuous mixing to yield niosomes proconcentrate on cooling. This proconcentrate was further hydrated with enough MQ water to maintain the total lipid or surfactant concentration at 10 mg/ml in the final formulation. This produced crude niosomes dispersions were left to swell for an overnight. The vesicle size was reduced by bath sonication for 1 hour. The sonicated vesicles were subjected to dehydration by freeze drying. The dried forms of niosomes were reconstituted with known volume of aqueous plasmid solutions based on predetermined N/P ratios.

Results: Up to N/P ratio of 10, no significant binding has been detected in the nisomoal system. This is likely attributed to the fact that the nisomoal formulations contain only one protonable amine per mole. The local cationic density provided by the delivery system is too low to complex plasmid DNA by electrostatic interaction at low N/P ratios.

Conclusion: Direct reconistitution of DNA solution and freeze-dried niosomes is not efficient to encapsulate plasmid DNA. Our future direction is to increase the N/P ratio and calculate the 50% binding and to freeze-dry the premixed DNA/Niosome solutions then reconstitute the lyophilized powder to allow for physical encapsulation along with electrostatic interaction.

Acknowledgment: Project was supported by Center of Excellence in Biotechnology Research, King Saud University, Riyadh, Saudi Arabia.

140. Two Release-Rate Monolithic System for Drug Controlled Release: *In vitro/in vivo* Evaluation

Tien Canh Le[1], Maxillien Arella[1,2] and Mircea-Alexandru Mateescu[1]. [1]Department of Chemistry and Centre BioMed, Université du Québec à Montréal, Montréal (Québec), Canada; [2]Spencer Pharmaceutical Inc.

Purpose: Recent evidence of cardiovascular risks associated with mostly non-steroidal anti-inflammatory drugs (NSAIDs) was reported by several studies, included Ibuprofen when given in elevated doses of more than 1200 mg/day. In order to reduce these adverse effects, numerous controlled release formulations such as biphasic, microsphere or multi-particulate devices were developed. However, these systems are generally expensive, required special equipments with multiple manufacturing steps, and often involved a large number of excipients including use of solvents. In this report, a new technology «Two Release-Rate (2RR) Monolithic Tablet» was developed for poorly soluble drug controlled release, particularly NSAIDs. This technology is mainly based on a calcium carboxymethyl-starch (CaCMS) complex which is low cost, simple to obtain and easy to manufacture by direct compaction.
Methods: Ibuprofen used as drug model, 2RR monolithic tablets with different potencies (400 and 600 mg) were obtained by direct compaction (2.3 T/cm^2) of a homogenous mixture of CaCMS complex and drug powders. The *in vitro* dissolution assay was conducted in simulated gastric fluid (pH 1.5) for 2 h and then in simulated intestinal fluid (pH 6.8) using an USP paddle (apparatus II) method. At predetermined time intervals, the ibuprofen release from tablet in was measured at 221 nm.
The *in vivo* study was carried out on the male dogs (*Canis familiaris*). After receiving treatments by oral administration, blood samples were collected in heparinized tubes and the Ibuprofen concentrations were determined using a liquid chromatography with tandem mass spectrometry method.
Results: *In vitro* dissolution assay of CaCMS formulation shows that there are two distinctive release rates: i) an initial fast release of ibuprofen approx. 200 mg similarly with the effective dose observed from the conventional immediate release form (Motrin® 200 mg) within 30 minutes in simulated gastric fluid; ii) a slow release of remaining doses for a longer period over 8 h.
For i*n vivo* study on Beagle dogs, pharmacokinetic parameters of the new controlled release formulation for single dose ibuprofen (400 mg) are near equivalence with multiple doses (3 tablets of Motrin® 200 mg Ibuprofen) of conventional formulation Motrin®.
Conclusion: An evident reduction of administered dose was noted for the new formulation. Although the present study for instance was limited to NSAIDs, the application for other active pharmaceutical ingredients is possible. It can be particularly useful for neurodegenerative diseases (*i.e*. Alzheimer) or mental disorders (schizophrenia), because patients are unable to follow frequent medications. In this case, 2RR formulation becomes necessary for patient compliance.

141. Tamoxifen-Loaded Novel Liposomal Formulation: Evaluation of Anti-Cancer Activity on DMBA-TPA Induced Mouse Skin Carcinogenesis

Amit Bhatia[1*], Bhupinder Singh[2], Om Prakash Katare[2]. [1]School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India. [2]University Institute of Pharmaceutical Sciences-UGC Centre of Advanced Study, Panjab University, Chandigarh, India.

Purpose: Tamoxifen (TAM) is a non-steroidal estrogen receptor modulator widely employed in the treatment of breast cancer. However, this drug is associated with serious side effects primarily due to its systemic distribution in body. Hence, the localized delivery of this drug would be highly beneficial. In the current studies, an endeavor has been made to investigate the efficacy of vesiculated TAM employing skin cancer model.
Methods: Phospholipid-based flexible liposomal gel of TAM was developed and employed for the evaluation vis-à-vis conventional gel. The pharmacodynamic activity was evaluated by employing two-stage cancer model i.e., dimethylbenzanthracene & tetradecanoyl phorbol acetate induced skin cancer in mice. Incidence of papillomas and histopathology evaluations were employed to determine the efficacy of the tested formulations.
Results: TAM lipogel significantly delayed the incidence of tumor (i.e., 59.4%) compared to conventional hydrogel gel (i.e., 31.5%) after treatment of 15 weeks. Significant decrease in the

incidence of tumors was seen with TAM lipogel (i.e., ~ 3 fold) compared to the tested gel at the completion of study. Histopathology studies revealed the superior activity of encapsulated drug. The tested formulation is devoid of any apparent adverse effect on skin. The results demonstrated carrier-dependent strong inhibition of skin carcinogenesis with encapsulated drug vis-à-vis drug in hydrogel.

Conclusion: The encouraging findings from the current work construe immense potential of the vesicular systems in the treatment as well as chemoprevention of cancer. Accordingly, it can be concluded that the phospholipid-based vesicular systems hold appreciable potential in ameliorating safety and efficacy of TAM using topical route of administration.

CSPT Posters - Day 2

Thursday, June 14, 2012

142. Sulphamethoxazole-hydroxylamine Reduces Levels of Peroxiredoxin 1 in Jurkat T Cells Expressing the HIV-1 Tat Protein

Adeyanju K, Bend JR, Rieder MJ, Dekaban GA (University of Western Ontario)

Treatment of HIV infection requires antiretroviral agents as well as drugs such as the antimicrobial Sulphamethoxazole (SMX), which is used as prophylaxis and first line therapy for Pneumocystis pneumonia, a common AIDS-defining disease. Hypersensitivity adverse drug reactions (ADRs) to a variety of drugs are common in HIV-infected individuals and cause significant morbidity with SMX remaining a major culprit. While the pathophysiology of drug hypersensitivity remains incompletely understood, ADRs to SMX have been linked to a reactive metabolite SMX-HA (SMX-hydroxylamine) which acts as a hapten following covalent conjugation with cellular proteins. We have also shown that the HIV-1 Tat protein contributes to ADRs in the HIV population. As the formation of hapten-protein conjugates is exacerbated by oxidative stress, we sought to elucidate the effects of Tat on the cellular redox proteome. We performed redox 2D gel electrophoresis, which enabled us to distinguish between thiol protein targets, using Tat-expressing Jurkat T cells in the absence and presence of SMX-HA. Exposure of the Tat-expressing cells to 200μM SMX-HA, led to a 2- to 3-fold increase in thiol protein oxidation as well as a significant decrease in the protein level of peroxiredoxin 1 compared to both the parent and HIV infected cell lines. This decline of peroxiredoxin 1 protein is indicative of significant oxidative stress that in turn lead to increased apoptosis.

143. Pharmacogenomic Diversity within African Populations and between the African and European Ancestries

Aminkeng F, Ross CJD, Sistonen J, Rassekh RS, Visscher H, Tishkoff S, Carleton BC, Hayden MR (University of British Columbia)

Rationale: Genetic diversity influences drug response and risk of adverse drug reactions (ADR). Therefore, it is important to characterize genomic variations at drug response loci by population.
Aims: 1) Characterize key pharmacogenomic variations in drug absorption, distribution, metabolism and excretion (ADME) genes in ethnically diverse African populations; 2) explore differences between the African and European ancestries and their relevance to drug metabolism/toxicity.
Study Population/ Methods*:* 281 Africans (AFR) and 907 Europeans (EUR) genotyped for 4536 SNPs in over 300 key ADME genes. Genetic ancestry ascertained by principal component analysis.
Results: Frequencies of risk ADR-associated SNPs were dramatically increased in AFR vs EUR, consistent with the known increased in the incidence of these ADRs in AFR. Cancer pharmacogenomics: *UGT1A6* rs17863783 for anthracycline-induced cardiotoxicity - P = 5.98E-019, 12.68% AFR vs. 2.48% EUR and *TPMT* rs1800462 (*TPMT* *2) for thiopurine toxicity - P = 1.34E-53, 15.20% AFR vs 0.33% EUR. Antiretroviral drug toxicity associated SNPs: *CYP2B6* rs34097093 for Efavirenz – P = 1.24E-300, 34.19% AFR vs 0.17% EUR; *CYP3A* rs2740574 for Indinavir - P = 6.65E-229, 37.99% AFR vs 2.98% EUR and *ABCC2* rs8187710 for Lopinavir – P = 1.50E-025, 21.25% AFR vs 5.47% EUR. Variations were also observed within AFR populations.
Discussion/Conclusion*:* Increased frequency of risk ADR-associated SNPs in Africans could explain the increased incidence of ADRs.

144. Mitochondrial DNA Replication Fidelity Protects Against Rotenone Toxicity

Fernando Bralha, Rebecca R. Laposa (University of Toronto)

Background/Objectives: A wide variety of structurally disparate drugs and environmental chemicals impair mitochondrial function. Since mitochondrial genome integrity maintains the function of the electron transport chain, we examined the role of the mitochondrial DNA polymerase gamma (POLG) in the cellular sensitivity to the classic mitochondrial toxin rotenone. Genetic variants of POLG are common in the human population.
Methods: Cells from mice with a proofreading-defective version of Polg and consequently abundant random point mutations in mitochondrial DNA were challenged with rotenone that reduced survival and proliferation of Polgm/m mouse embryonic fibroblasts (MEFs). MEFs were forced to rely on mitochondrial oxidative phosphorylation rather than glycolysis for ATP production by substituting galactose for glucose culture media.
Results: Rotenone decreased ATP levels and proliferation in Polgm/m MEFs more than wild type, suggesting that Polgm/m MEFs decreased proliferation in order to maintain intracellular ATP levels. The level of DNA replication-blocking damage and mitochondrial DNA copy number were higher in Polgm/m MEFs than wild type and unaltered by rotenone.
Conclusions: We conclude that mitochondrial genomic stability mediated by POLG indirectly protects against rotenone-initiated mitochondrial dysfunction and cellular toxicity. This model may be useful for toxicity screening of drugs and chemicals that inhibit the mitochondrial electron transport chain.

145. Placental ABC Efflux Transporter Expression in Insulin-managed Diabetes

Cressman AM, Anger GJ, Piquette-Miller M (University of Toronto)

Drug efflux transporters in the placenta can significantly influence the materno-fetal transfer of a diverse array of drugs and other xenobiotics. To determine if clinically important placental drug efflux transporter expression is altered in pregnancies complicated by insulin-managed gestational diabetes mellitus (GDM-I) or insulin-managed type 1 diabetes mellitus (T1DM-I), we compared the expression of multidrug resistance protein 1 (MDR1), multidrug resistance-associated protein 2 (MRP2) and the breast cancer resistance protein (BCRP) via western blotting and quantitative real-time polymerase chain reaction in samples obtained from insulin-managed pregnancies to healthy term-matched controls. At the level of mRNA, we found significantly increased expression of MDR1 in the GDM-I group compared to both the T1DM-I ($p < 0.01$) and control groups ($p < 0.05$); however significant changes in the placental protein expression of MDR1, MRP2 and BCRP were not detected ($p > 0.05$). Interestingly, there was a significant, positive correlation observed between plasma hemoglobin A1c levels (a retrospective marker of glycemic control), BCRP protein expression ($r = 0.45$, $p < 0.05$) and BCRP mRNA expression ($r = 0.58$, $p < 0.01$) in the insulin-managed DM groups. Collectively, the data suggest that the expression of placental efflux transporters is not altered in pregnancies complicated by diabetes when hyperglycemia is managed; however, given the relationship between BCRP expression and plasma hemoglobin A1c levels it is plausible that their expression could change in poorly managed diabetes. Further studies are required.

146. Potential Role of 4-hydroxy-2-nonenal in the Development of Nitrate Tolerance

D'Souza YP, Bennett BM (Queen's University)

Background: Tolerance to organic nitrates such as glyceryl trinitrate (GTN) is associated with a loss of vasodilator activity, oxidative stress, and inactivation of aldehyde dehydrogenase 2 (ALDH2). 4-hydroxy-2-nonenal (4HNE) is a by-product of oxidative stress-induced lipid peroxidation and this toxic aldehyde can form adducts with proteins, resulting in cell dysfunction. ALDH2 plays a major role in the detoxification of 4HNE. Taken together, these findings raise the possibility that tolerance development could be mediated by increased levels of 4HNE resulting from GTN-induced inactivation of ALDH2. In the present study we used an *in vivo* GTN tolerance model and a cell culture model of nitrate action to assess whether GTN exposure resulted in HNE adduct formation, and whether exogenous 4HNE affected GTN-induced relaxation

and cGMP accumulation.
Results: In aortae from rats treated with GTN (0.4 mg/hr for 2 days) or in porcine kidney epithelial cells (PK1) incubated with 1μM GTN, a marked increase in HNE-protein adducts was observed. Preincubation of PK1 cells with HNE (10, 30 and 100μM) resulted in a dose-dependent decrease in GTN-induced cGMP accumulation, whereas the cGMP responses to the NO donor, DEA/NO,were unaffected. Preincubation of isolated rat aorta with HNE (0.1, 1 and 10μM) resulted in a dose-dependent decrease in vasodilator responses to GTN, thus mimicking GTN-tolerance.
Conclusions: Together, these results indicate an association between GTN-induced HNE adduct formation and decreased responses to GTN, suggesting a role for HNE in the development of GTN tolerance.

147. Validation of the *in vitro* Platelet Toxicity Assay (*i*PTA) for the Diagnosis of Suspected Hypersensitivity Reactions to Sulfonamides

Elzagallaai AA, Rieder MJ, Koren G (University of Western Ontario)

Drug hypersensitivity syndrome (DHS) is a rare but potentially adverse drug reaction (ADR). A valid diagnostic test for DHS would be a major advance in patient care and evaluation of possible ADRs during drug development and clinical trials. We have recently developed a novel diagnostic test for DHS called the in vitro platelets toxicity assay (iPTA) using peripheral blood platelets as a surrogate cell model as oppose to the traditional lymphocyte Toxicity Assay (LTA) that uses peripheral lymphocytes. This work was to validate the use of the iPTA for the diagnosis of DHS. Forty-seven individuals (25 DHS patients and 22 healthy controls) were recruited to participate in this research. 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. There was concentration-dependent toxicity in the cells of patients in both the LTA (lymphocytes) and iPTA (platelets) and toxicity was greater in cells from patients thn from controls. The iPTA was significantly more sensitive than the conventional LTA test in detecting the susceptibility of patient cells to in vitro toxicity. The novel iPTA has considerable potential to be used as an investigative tool for DHS.

148. Meta-analyses of Small Cohort Studies in Teratology – Do they Predict Later Results of Large Cohorts?

Etwel F, Walfisch A, Abuzgaia A, Koren G (University of Western Ontario)

Introduction: Meta-analyses have become increasingly useful in the area of clinical teratology. Observational studies provide an important and often solitary source of information to be included in such meta-analyses. However, the quality of published meta-analyses of small observational studies is variable.
Objective: The aim of the study was to examine the validity of the conclusions reached by meta-analyses of small cohort studies. These results were compared to more recent, much larger database-derived cohort studies.
Methods: All meta-analyses published in peer review journals by the Motherisk Program were identified. The results of these meta-analyses were compared to those of large cohort studies published at later dates.
Results: Out of about 60 meta-analyses published by Motherisk on medicinal drugs between 1985 and 2011, 9 different meta-analyses were successfully matched to large cohort studies published later on the same exposure. There were 7 “negative” meta-analyses (showing no teratological effects) and 2 “positive” ones. In all nine instances, the meta-analyses accurately predicted the results of the later, large cohort studies.
Conclusion: Meta-analysis of smaller studies generates the correct signal in estimating human teratogenicity years before a large and methodologically superior cohort studies are published.

149. Relationship Between Meconium fatty Acid Ethyl Esters and Measures of Structural and Functional Toxicity in the Fetal Guinea Pig Following Chronic Maternal Ethanol Administration

Gareri JN, Hewitt AJ, Reynolds JN, Brien JF, Koren G (Hospital for Sick Children)

FAEE are ethanol metabolites present in high concentrations in the meconium of prenatally ethanol-exposed human neonates. This study examines associations between gestational age, hippocampal weight, CYP2E1 activity, HPA-axis function, and FAEE in the meconium of ethanol-exposed third-trimester equivalent fetal guinea pigs. Pregnant guinea pigs were randomized into two groups; ethanol-exposed (4g/kg/day, n = 12) and isocaloric-sucrose pair-fed controls (n = 12). On gestational day (GD) 45, four animals from each group were euthanized. Littermates were euthanized and hippocampi were dissected and weighed. Plasma was analyzed for ACTH and cortisol. Fetal liver was excised; mitochondrial and microsomal fractions were assessed for CYP2E1 activity. Meconium was collected and analyzed by GC-MS; ethyl palmitate, stearate, oleate, and linoleate were quantified. This was repeated at GD 55 and GD 65. Negative correlations were found between FAEE and hippocampal weight (Spearman r = -0.549; p = 0.003) as well as microsomal CYP2E1 activity (Spearman r = -0.799, p = 0.002) at GD 65. A positive correlation was found between mean littermate FAEE and total maternal ethanol dose. These data indicate that meconium FAEE constitute a biomarker of hippocampal injury and cumulative ethanol exposure. The negative correlation between fetal hepatic CYP2E1 activity and meconium FAEE indicates that FAEE production in the fetus may be inversely dependent upon fetal oxidative metabolism capacity.

150. Pharmacological Blockade of the Androgen Signaling Stimulates Androgen Glucuronidation in Prostate Cancer Cells

Grosse L, Paquet S, Fazli L, Belanger A, Rennie P, Barbier O (Laval University)

Androgen receptor (AR) activation is a crucial event for both prostate cancer (PCa) initiation and progression. An efficient way for androgen inactivation in prostate cells consists in their conjugation with the highly hydrophilic glucuronide moiety. This reaction, catalyzed by the UDP-glucuronosyltransferase (UGT)2B15 and UGT2B17 enzymes, produces inactive and easily excretable glucuronide derivatives in the human prostate. AR was previously identified as a negative regulator of UGT2B15 and UGT2B17 genes expression. Based on these observations, ex vivo and in vivo experiments were performed to test the possibility that clinically used anti-androgens may affect this AR-dependent down-regulation. Using the PCa cell models LNCaP and LAPC-4, we show that AR antagonist Casodex causes a time- and dose-dependent induction of UGT2B15 and UGT2B17 genes expression, as well as an improved androgen glucuronidation. The contribution of AR in these regulatory events was confirmed using LNCaP cells knock-downed for AR. In addition, tissue microarray experiments demonstrated that PCa samples from patients exposed to neoadjuvant hormonal therapy exhibited increased UGT2B15 protein levels. UGT2B17 levels were transiently increased in patients treated for up to 5 months. Overall, these observations illustrate an unexpected anti-androgenic effect for the pharmacological blockade of the androgen signaling in prostate cancer cells.

151. Azole-based Heme Oxygenase Inhibitors and their Effects on Breast Cancer Growth and Metastasis in vivo

Hum M, Dercho RA, Vlahakis JZ, Szarek WA, Nakatsu K (Queen's University)

Heme oxygenase (HO) is responsible for the breakdown of heme to biliverdin, free iron, and carbon monoxide (CO). The two major isoforms, HO-1 (inducible) and HO-2 (constitutive) are involved in a variety of physiological functions, including apoptosis, inflammation and angiogenesis - important to the progression of some cancers. Previous work using metalloporphyrin-based compounds has identified HO inhibition as a potential target for the prevention of tumour growth and progression. These compounds are limited by their lack of selectivity for HO. Novel azole-based HO inhibitors have demonstrated increased in vitro selectivity for HO and were tested for their effects on breast cancer growth & metastasis and cancer-related angiogenesis. *In vitro* experiments included observing the effects on AC2M2 cell viability and

endothelial tube growth in an aortic ring model. *In vivo* experiments involved the implantation of GFP-labelled AC2M2 cells into the mammary fat pad of female nude mice, which then received metronomic treatment with an azole-based compound or vehicle. Primary tumours were removed on day 21 and metastases were allowed to grow for another 10 days. We hypothesized that the azole-based HO inhibitors would decrease primary tumour volume, decrease the incidences of lung metastases and that resulting secondary tumours would be smaller in size compared to control. The findings will help determine whether HO is an appropriate target in the treatment of breast cancer.

152. Preventing Asthma Exacerbations with a Short Course of Oral Steroids at the Earliest Signs of Upper Respiratory Tract Infection: Preliminary Results of an Ongoing Policy Trial

Jimenez-Mendez R[1,2,3], Smith A[1,2,3]; Mitton C, Goldman R[1,4], Carleton B[1,2,3] (University of British Columbia, BC Children's Hospital, [3]Child & Family Research Institute)

Background: Up to 80% of asthma exacerbations are caused by upper respiratory tract infections (URTIs). Despite recommendations in guidelines, prescription of a short course of oral steroids at the earliest signs of URTI is not widely accepted. We are conducting a policy trial to determine barriers to this practice and patient outcomes of this intervention.
Methods: Patients with two or more URTI-related ED-visits in the past year were recruited into the trial upon diagnosis of asthma exacerbation. Patients receive a dispensed prescription of oral steroids with detailed instructions to use the medication at the earliest sign of URTI. A second, matched group of patients follow the standard of care. Patients' outcomes are collected for one year from follow-up interviews on quality of life, asthma exacerbation management, URTI signs, symptoms and frequency, ED use, as well as health provider's acceptance of this practice.
Results: We enrolled 97 patients; 27 in the intervention arm. Several factors have been identified as barriers for health providers to completely accept this preventative drug prescribing: the belief that families will not be able to correctly use the oral steroid at the time of URTI onset, that oral steroids will be administered but the child will not receive medical follow up, and that there is questionable value of giving a medication for future exacerbations.
Conclusions: We have been able to document the value of this intervention as well as some of the barriers healthcare providers have in order to completely accept this intervention. Next steps will be to conduct focus groups with healthcare providers and families of patients to further understand barriers of intake.

153. Methadone Pharmacokinetics in Opiate Dependent Patients

Kapur B (The Hospital for Sick Children)

In Opioid Dependence programs treatment with Methadone has been successful. Although the patient is on a maintenance dose they often ask for re-adjustment of their dose. Almost all of these patients have co-morbidities and require additional medication so drug-drug interaction cannot be ruled out. Methadone half-life is the functionalmarker of drug-drug interactions and reflect the state of methadone's metabolism.
Method: Pre-dose and post dose blood samples, dose, weight and patient's medication list was obtained. The assays for methadone and metabolite were done by immunoassay that had previously been validated against both HPLC and GC. We calculated t½, Cl and Vd for both methadone and its metabolite.
Result: From 2002 to Jan 2012, 239 patient samples were analysed. Since 2006 on 65 patients we also had the list of medications. Six of these had no other medication and the 59 remaining had received an average of 3.5 medications (range 1 to 13). These included substrates and inhibitors of methadone. Methadone: Half-life could be calculated on 214 patients t½ ranged from 6.6h to 167h. Removing extreme values <15h to >50h, mean 27.9h, n=177 was obtained. Half-life correlated significantly with dose in mg/kg, weight and pre-dose methadone blood levels at r=-1954, $p<0.032$; r= .3612, p=000 and r=.5939, p=0.000 respectively.
Conclusion In this large series changes in methadone half-life in many patients could be attributed to the medication the patient was prescribed.

154. Regulation of Expression and Transport Function of Organic Anion Transporting Polypeptide 2B1 Transcriptional Start Site Variants

Knauer MJ, Girdwood AJ, Kim RB, Tirona RG (University Western Ontario)

Background: Organic Anion Transporting Polypeptide 2B1 (OATP2B1) is a membrane transporter that facilitates the cellular uptake of numerous endogenous compounds and drugs. 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 and promoters but share common subsequent exons. These splice variations are expected to encode either a full length or truncated protein. Since little is known about OATP2B1 splice variants we investigated the transport function and relative expression in key tissues responsible for drug absorption and elimination.
Methods and Results: Both the predicted full length and truncated forms of OATP2B1 were detected using variant-specific PCR in liver and small intestine, albeit in differing proportions. The transcriptional activity and regulation of the truncated variant SLCO2B1 gene promoter was examined by dual luciferase reporter assay and ChIP. We determined that HNF4α was able to transactivate the truncated variant promoter but not the full length. Transport kinetics were determined after heterologous expression in cultured cells, demonstrating that the truncated variant was capable of transporting the known OATP2B1 full length variant 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.

155. The Free Radical Spin Trap α-phenyl-N-t-butylnitrone (PBN) Reduces Postnatal Cognitive Deficits Caused by in utero Exposure to Methylmercury

Lam KC, Wells PG (University of Toronto)

Methylmercury (**MeHg**) causes neurodevelopmental deficits in infants, possibly in part via the formation of reactive oxygen species (**ROS**). ROS-mediated oxidative damage to cellular macromolecules like DNA has been implicated in embryopathies caused by several xenobiotics. We previously found that fetuses exposed to a single maternal dose of 4-8 mg/kg MeHg chloride exhibited a dose- and time-dependent increase in oxidatively damaged DNA in fetal brain. Herein we investigated the role of ROS in postnatal cognitive deficits in CD-1 mice caused by in utero exposure to the lower dose of MeHg. Pregnant dams were injected i.p. on gestational day (**GD**) 17 with 4 mg/kg MeHg or its phosphate-buffered saline vehicle, with or without pretreatment with the free radical spin trap α-phenyl-N-t-butylnitrone (**PBN**). The offspring were evaluated in the object recognition test (**ORT**) at 6 weeks of age, or in the passive avoidance test (**PAT**) at 4 months, and using von Frey filaments at 6 weeks and 4 months of age. MeHg caused cognitive deficits in both the ORT ($p < 0.05$) and PAT ($p < 0.05$). No change in mechanosensitivity was observed in the von Frey test, confirming that PAT results were not confounded by reduced sensory function. Pretreatment with PBN reduced MeHg-initiated deficits in both the ORT ($p<0.05$) and the PAT, with no effect in the von Frey test, implicating ROS in the mechanism of MeHg-initiated cognitive deficits, to which the developing fetal brain is highly sensitive.

156. Drug-Transporter Interactions: Inhibition of MCT1 and MCT4 by Statins and Other Acidic Drugs

Leung Y, Lu J, François B, Turgeon J, Michaud V (University of Montreal)

Background: The muscle injury, also called myopathy, is a well known side effect associated with statin medications. In addition to statins, many drugs have been implicated as causes of myopathies and in some more serious cases, rhabdomyolysis. We hypothesized that these side effects could be related to an intracellular accumulation of lactic acid. Our objective was to use cell models expressing monocarboxylate transporters, MCT1 and MCT4, to determine whether the use of statins and other acidic drugs can inhibit the transport of lactic acid.
Methods: Cell lines used as models for MCT1 and MCT4 were Hs578T and MDA-MB-231, respectively. The cells were incubated with [^{14}C]lactic acid at 37°C and the intracellular concentration of radioactive lactic acid was measured. Inhibition studies were conducted by co-

incubating the cells with different concentrations of acidic drugs and lactic acid.
Results: 15 drugs have been tested for the inhibition of MCT1 and MCT4. Among these drugs, atorvastatin, irbesartan showed the highest inhibition with IC_{50} of 20-50 μM; losartan, valsartan showed an intermediate inhibition with IC_{50} of 200-500 μM; and salicylic acid showed a lower inhibition with IC_{50} >1000 μM.
Conclusions: These results imply that atorvastatin and other acidic drugs can lead to the accumulation of lactic acid due to the blockage of MCT1 and MCT4. Further studies are required to link the intracellular accumulation of lactic acid to the muscle pain.

157. Tricyclic Drugs Inhibit the Uptake of Rosuvastatin through the OATP1A2 Transporter

Lu J, Guilarte Moya L, Leung Y, Gaudette F, Turgeon J (University of Montreal)

Background: OATP1A2 is a membrane transporter involved in the absorption of various drugs. Previous results from our group demonstrated that the uptake of rosuvastatin through OATP1A2 can be inhibited by several β-blockers, where carvedilol is the most potent inhibitor. Carvedilol structurally differs from the other β-blockers tested by its tricyclic moiety. The goal of this study was to determine whether the tricyclic structure of carvedilol is responsible for its strong inhibitory effect on OATP1A2.
Methods: A HEK293 cell line overexpressing OATP1A2 was used as model for the study. First the cells were grown to confluence on 12-well plates. Then, they were co-incubated in the presence of rosuvastatin and increasing concentrations of different tricyclic drugs: amitriptyline, carazolol, carbamazepine, carbazole, chlorpromazine, imipramine, and phenothiazine. The amount of rosuvastatin transported in the cells was measured by UV-HPLC.
Results: The tricyclic drugs carazolol, amitriptyline, imipramine, and chlorpromazine inhibited rosuvastatin uptake through OATP1A2 with IC_{50} of 4.0, 5.0, 8.0, and 29.6 μM, respectively.
Conclusions: This study shows that the inhibitory component is made up of the tricyclic ring with a short carbon chain and that a tricyclic ring alone is not enough to inhibit OATP1A2 transport. Consequently, drugs composed of a tricyclic ring with a short carbon chain may strongly modulate the transport of OATP1A2 substrates.

158. Pregnancy Outcome and Child Neurodevelopment Following in utero Exposure to Maternal Cancer

Nulman I, Barrera M, Maxwell C, Koren G (The Hospital for Sick Children)

Background: Limited data exists on cancer-complicated pregnancy outcomes and management guidelines are inconsistent.
Objectives: This report outlines existing knowledge of perinatal cancer and presents pediatric and neurodevelopmental outcomes of children exposed in utero to maternal malignancy.
Methods: 24 children (aged 3-12) prenatally exposed to maternal malignancy were assessed. Information on maternal malignancy and pediatric outcomes was documented. Children's neurodevelopment was assessed using standardized psychological tests.
Results: 15 children were exposed to chemotherapy and/or radiation. 9 children exposed to maternal cancer or surgery served as controls. Control children had shorter gestations (37.2 wks vs 35.2 wks) and lower birth weights (3115 gm vs 2600 gm). Children from both groups were similar in their developmental milestones; anthropometric measurements; Full-scale, Verbal, and Performance IQs; and CBCL scores at testing.
Conclusions: Child's physical and neurological development was within population norms for both groups. Shorter gestations and low birth weights among controls were due to planned deliveries in order to start treatment. Prematurity is associated with increased child morbidity and mortality and should be minimized. These results should be considered when weighing the benefits of timely versus postponed maternal treatment. More research is needed to support these results and ensure optimal maternal treatment and fetal safety.

159. Testing Knowledge Acquisition in Nurses and Parents from Educational Tools about Managing Pain during Childhood Immunization

Parikh C[1], Smart S[1], Shah V[2], Wang J[1], Ipp M[3], Leung E[4], Hetherington R[3], Pillai-Riddell R[5], Sgro M[4], Franck L[6], Taddio A[1]. ([1]University of Toronto, Toronto, [2]Mt. Sinai Hospital, [3]The Hospital for Sick Children, [4]St. Michael's Hospital, [5]York University, [6]University of California San Francisco)

Background: An evidence-based clinical practice guideline (CPG) for managing childhood vaccine injection pain was recently published (CMAJ 2010).
Objective: To evaluate parents' and nurses' knowledge about effective pain management (PM) methods before and after exposure to educational materials (pamphlet and video) developed from the CPG.
Methods: Participants included 29 nurses [mean age (SD) in years, 40(13)] and 37 parents [33(4)] on the postnatal ward of Mount Sinai Hospital in Toronto. Participants completed the same knowledge test containing 10 true/false questions regarding the effectiveness of various PM methods. Percent correct scores were compared within groups using RM ANOVA. Only answers whereby participants reported both the correct response and complete certainty in their level of confidence regarding their response were included as correct.
Results: The mean percent correct scores at baseline, post-pamphlet, and post-video were 20% (SD=19), 61% (21), and 72% (16) for parents and 34% (18), 64% (21), and 70% (15) for clinicians. Statistically significant ($P<0.01$) increases in knowledge were observed at each level of intervention for both groups.
Conclusions: This study provides evidence of knowledge acquisition from the educational pamphlet and video. Importantly, that knowledge was further increased by the video after reading the pamphlet suggests that both should be used together for future vaccine pain management education.

160. Normal Subjects Exposed to Nifedipine via Differing Osmotic Delivery Systems have Differing Patterns of Nocturnal Dipping

Pollak PT, Herman RJ, Zarnke KB (University of Calgary)

Several patients with unexplained increases in BP >10 mmHg were observed following silent switching of nifedipine from a 2-compartment (Adalat XL=AdN) to a 1-compartment osmotic delivery system (Mylan=MyN). To explore potential differences, we obtained 24-h ABPM recordings in 3 healthy, normotensive subjects. Recordings were made for each formulation on Day 7 of 30 mg/d dosing. Delay in nocturnal dipping to 85 mmHg was observed for MyN in all 3 subjects.
Table. Subj: Nocturnal Dip Delay, 24-h mean MAP's for MyN vs and A: 5 h, 99 ± 09 vs 94 ± 10, B: 1 h, 98 ± 07 vs 95 ± 10, C: 4 h, 99 ± 10 vs 93 ± 15
Clinical dosing of nifedipine produces concentrations on the steep portion of the dose-response curve and its T1/2 is only 2 h. Therefore, changes in delivery rate during a partial dosing interval (2-4 h) would be expected to change BP response, even though differences in hourly concentrations might not be detected on 24-h AUC's. Despite being deemed bioequivalent (24-h AUC/Cmax diff ≤ +/- 20%), differering release technologies have differences in time-release profiles. This is most clearly apparent in healthy subjects taking no other medications, as seen in these preliminary data. Clinically, some patients also appear sensitive to differences in nifedipine delivery formulations. These preliminary data in 3 subjects suggest that nocturnal dipping should be scrutinized on 24-h ABPM pressures if there is a question that patients have different responses to nifedipine formulations.

161. 5-bromo-2'-deoxyuridine (BrdU) Rescues Neurons from DNA Damage-initiated Apoptosis

Rajakulendran N, Tamblyn L, Laposa R (University of Toronto)

Background/Objectives: DNA damage in neurons can be lethal. The protective neuronal DNA damage response (DDR) can involve cell cycle entry of a postmitotic neuron. While investigating neuronal DDR, we observed that BrdU, a thymidine analogue

that incorporates into new DNA during DNA replication, dramatically prevented neuronal death after UV-initiated DNA damage. Hypothesis: BrdU protects neurons from death following DNA damage by modulating the neuronal DNA damage response.
Methods: Primary mouse cortical neuronal cultures from E16.5 mice were used. The DNA damaging agent was UV light (254nm). To quantify apoptosis, flow cytometry with Annexin V and immunocytochemistry with caspase-3 was utilized. Hoechst staining was utilized to determine the DNA content of neurons.
Results: In the absence of BrdU, neuronal apoptosis following 10J/m2 UV was 26% (±2.6) while the addition of BrdU (30uM) significantly reduced neuronal apoptosis to 15% (±2.7) ($p<0.001$) 24h after UV-damage. UV damaged (10J/m2) neurons were treated with BrdU (1-30uM) for 24h. A BrdU concentration as low as 0.1uM decreased neuronal apoptosis and 10uM was optimal. We assessed cell type specificity of BrdU protection; BrdU does not rescue mouse embryonic fibroblasts. BrdU neuroprotection was independent of BrdU labeling of DNA. UV increased neuronal DNA content, indicative of cell cycle re-entry. Thymidine and additional halogenated thymidine analogues CldU and IdU all rescued UV-damaged neurons to varying degrees. We investigated the neuronal DNA damage response and BrdU does not alter UV-induced p53 expression or global DNA methylation.
Conclusions: This is the first report of neuroprotective properties of thymidine analogues, suggesting a novel mode of neuroprotection against DNA damage.

162. Aryl Hydrocarbon Receptor-dependence of Dioxin Effects on Constitutive Mouse Hepatic Cytochromes P450 and Growth Hormone Signaling Components

Riddick DS, Lee C (University of Toronto)

Background: The aryl hydrocarbon receptor (AHR) mediates most responses to the pollutant, 2,3,7,8-tetracholorodibenzo-*p*-dioxin (TCDD). A readily metabolized AHR agonist, 3-methylcholanthrene, disrupts mouse hepatic growth hormone (GH) signaling components and suppresses cytochrome P450 2D9 (*Cyp2d9*), a male-specific gene controlled by pulsatile GH via signal transducer and activator of transcription 5b (STAT5b).
Objectives: Using *Ahr* $^{-/-}$ mice as a model to examine AHR-dependence of responses, we studied effects of TCDD, a poorly metabolized AHR agonist, on hepatic expression of selected constitutive P450s, GH signaling components and STAT5b target genes.
Method: Male *Ahr* $^{-/-}$ and wild-type C57BL/6J mice received a single dose of TCDD (1000 µg/kg) or vehicle by gavage and liver was harvested 19 h later. Levels of mRNAs were assayed by RT-PCR.
Results: Two STAT5b target genes, *Cyp2d9* and major urinary protein 2 (*Mup2*), were suppressed by TCDD with AHR-dependence. TCDD also decreased GH receptor, Janus kinase 2, and STAT5a/b mRNA levels with AHR-dependence. Without triggering acute inflammation, TCDD caused AHR-dependent induction of *Cyp1a1* and NADPH-cytochrome P450 oxidoreductase (*Por*) and suppression of *Cyp3a11*. Basal mRNA levels for CYP2D9, CYP3A11, POR, serum amyloid protein P, and MUP2 were influenced by *Ahr* genotype.
Conclusions: AHR activation *per se* leads to dysregulation of hepatic GH signaling components and suppression of some, but not all, STAT5b target genes.

163. The Detection of Cortisol in Human Sweat: Implications for Measurement of Cortisol in Hair

Russell EW, Koren G, Rieder MJ, Van Uum S (University of Western Ontario)

Background: Hair cortisol analysis is an effective measure of chronic stress. Cortisol is assumed to enter the hair via serum, sebum, and sweat, however the extent to which sweat contributes to hair cortisol content is unknown.
Hypothesis: It was hypothesized cortisol would be found in sweat. Further, exposures to a hydrocortisone solution with a sweat-like cortisol concentration were hypothesized to affect hair cortisol concentrations, but would be normalized with washing.
Methods: Sweat and saliva samples were collected from 17 subjects, and analyzed by salivary ELISA. Subsequently, an *in vitro* test on hydrocortisone exposure was conducted. Residual hair samples were immersed in a 50ng/ml hydrocortisone solution for periods lasting 15 minutes to 24 hours, followed by a wash or no-wash conditions. Hair cortisol content was determined using a modified salivary ELISA protocol.

Results: Sweat cortisol concentrations were 74.62±41.51ng/ml (mean±SD) and ranged from 8.16-141.7ng/ml. Hair exposure to a 50ng/ml hydrocortisone solution for 60 minutes or more resulted in significantly increased hair cortisol concentrations ($P<0.01$). Washing did not affect immersion-increased hair cortisol concentration.
Conclusions: Human sweat contains cortisol that likely contributes to hair cortisol content. Subjects with prolonged sweating at the time of hair collection may have increased hair cortisol concentrations that cannot be decreased with conventional washing procedures.

164. Pharmacogenomics of Adverse Drug Reactions: Establishing Priorities for Research Programs

Shaw K, Amstutz U, Castro-Pastrana LI, Loo TT, Ross CJ, Hayden MR, Carleton BC (University of British Columbia, Child & Family Research Institute)

Background: The importance of genetic factors in the incidence and severity of many adverse drug reactions (ADRs) is being increasingly recognized. A tool was developed to facilitate the prioritization of drugs and their associated ADRs for pharmacogenomic studies.
Methods: Scores were based on 25 criteria that are relative for clinical and genetic research: pharmacoepidemiology of drug use and ADR prevalence, likelihood of genetic basis of the ADR, pharmacology of the drug and ADR mechanism, as well as study feasibility within a given research setting (required resources, patient recruitment, timelines). The tool was applied to five drug/ADR combinations by two researchers independently and scores were compared using the intraclass correlation coefficient (ICC).
Results: Total scores for target ADRs ranged from 33% (19.5/60) to 73% (44/60) of the maximum possible score. The tool performed as expected, with a frequently occurring and severe ADR previously studied receiving the highest score, while a rare ADR with difficult clinical characterization and a milder ADR scored lower. Good agreement was observed between the scientific, feasibility, and total scores from two reviewers (ICC values = 0.895, 0.980, and 0.983, respectively).
Conclusion: This tool allows the direct comparison of strengths and weaknesses of drug/ADR study targets and can be used by research teams to better understand which pharmacogenomic studies are best suited for their research environments.

165. Optimizing Periconceptional and Prenatal Folic Acid Supplementation: Steady State Red Blood Cell and Plasma Folate Levels Achieved with 5mg vs. 1.1mg Folic Acid in Prenatal Multivitamin Supplements among Pregnant Women

Shere M, Kapur B, O'Connor D, Koren G (The Hospital for Sick Children)

Background: Folic acid supplementation before and during pregnancy reduces the risk of neural tube defects (NTDs). Maximal protection against NTDs is achieved through maternal red blood cell (RBC) folate concentrations of 900nmol/L or greater.
Objectives: To compare the steady-state periconceptional and gestational RBC and plasma folate levels in women supplementing with prenatal multivitamins containing 1.1mg vs. 5mg folic acid.
Methods: 8 women, who were early in pregnancy or planning, and were not previously taking folic acid, were enrolled in the study after obtaining informed consent. Participants were randomized to either 1.1mg or 5mg of folic acid-containing multivitamins daily till 30 weeks gestational age. Plasma and RBC folate levels were measured at baseline, and at 6, 12 and 30 weeks of gestation using a competitive-binding receptor assay.
Results: No significant difference was observed between the baseline RBC concentrations of the 2 groups (baseline was 2849 ±143nmol/L and 2840 ±230nmol/L in the 1.1mg and 5mg groups respectively). However, differences were observed in RBC concentrations between the groups at 6, 12, and 30 weeks gestation: RBC folate concentrations by 30 weeks gestation were 4149 ±321nmol/L in the 1.1mg vs. 6175 ±394nmol/L in the 5mg group.
Conclusions: The use of 5mg folic acid produced and maintained higher blood folate concentrations compared with 1.1mg folic acid, thus rendering greater protection against NTDs.

166. Educating Parents about Pain Management during Immunization

Smart S[1], Parikh C[1], Shah V[2], MacDonald N[3], Taddio A[1]. ([1]University of Toronto, [2]Mt. Sinai Hospital, [3]Dalhousie University)

Background: A fact sheet for parents regarding effective pain management (PM) strategies for infants was developed from a recently published clinical practice guideline (CMAJ 2010).
Objective: To evaluate parents' self-reported knowledge and utilization of effective PM methods from the fact sheet.
Methods: A convenience sample of new mothers was recruited from the postnatal wards of two perinatal centres: Mount Sinai Hospital (MSH), Toronto and IWK Health Centre (IWK), Halifax). IWK was randomly allocated to passively distribute the fact sheet in parent hospital discharge packages. Participants were contacted 2-3 months after discharge and asked about knowledge and utilization of PM interventions.
Results: 86 and 92 parents from MSH and IWK, respectively, participated. Overall mean age was 32 years (SD=5); 66.4% were university educated. Knowledge was significantly increased for parents from IWK vs. MSH for PM methods, including: 1) sugar water (22.8% vs 11.6%), 2) local anesthetics 42.4% vs. 30.2%, and 3) parent behaviour; 90.7% vs. 97.8%); all $P<0.05$. Reported utilization did not differ; 2.17% vs. 0%, 1.09% vs. 0% and 98.9% vs. 97.7%, respectively (all $P>0.05$).
Conclusions: Passive dissemination of the fact sheet in discharge packages increased parental knowledge about PM methods at little cost. Knowledge is first step in behaviour change supplementing the fact sheet with other KT strategies is needed to influence parental behaviour.

167. Characterization of Aminosilane Coated Iron Oxide Nanoparticles for Brain Targeted Delivery

Sun Z, Yathindranath V, Chu S, Parkinson FE, Hegmann T, Miller DW (University of Manitoba)

Background: Aminosilane coated iron oxide nanoparticles (AmS-IONPs) have been widely used in constructing complex and multifunctional drug delivery systems. However, suitability of AmS-IONPs for brain related drug delivery applications is unknown.
Objectives: To determine AmS-IONPs toxicity and cell accumulation in brain related cell cultures and assess permeability across cell culture model of the blood-brain barrier (BBB).
Methods: AmS-IONPs were examined in a mouse brain microvessel endothelial cell line (bEnd.3) and mouse primary neurons and astrocytes. Cell accumulation of IONPs was examined using Ferrozine assay and cytotoxicity was assessed by MTT assay. Permeability of AmS-IONPs in bEnd.3 monolayer grown on PET membrane inserts (1 μm pore) was evaluated.
Results: Acute toxicity was observed in neurons and astrocytes above 70 ug/ml. Rank order of accumulation of AmS-IONPs was astrocytes> bEnd.3 cells> neurons. Presence of a magnetic field increased cell uptake but had minimal effect on AmS-IONP toxicity. Negatively charged AmS-IONPs showed 16% flux across bEnd.3 monolayers after 24 hrs with aid of magnetic field.
Conclusion: AmS-IONPs were well tolerated by all cells examined. Permeability of positively charged AmS-IONPs across confluent bEnd.3 monolayers was negligible. Modification of surface chemistry of the AmS-IONPs improved the permeability profile in cell culture model of the BBB. Therefore, AmS-IONP is a promising candidate for delivery of drugs into the brain.

168. Implementation of a Clinical Practice Guideline about Immunization Pain Management in a Public Health Setting

Taddio A[1,2], Chan S[3], McIntyre C[3], Deeter B[3], Pielak K[3] (University of Toronto, The Hospital for Sick Children, British Columbia Centre for Disease Control)

Objective: To evaluate the impact of a new evidence-based program guideline about pain management during childhood immunization injections on public health immunizers' attitudes, beliefs, and analgesic practices.
Method: In a controlled, before-and-after methodology, participating intervention group public health nurses (PHNs) were educated about pain-relieving strategies according to a new British Columbia Immunization Program Manual. Attitudes and beliefs of PHNs towards immunization injections and pain management and percentage of children receiving newly recommended pain-

relieving strategies during immunization visits were compared before and after intervention.
Results: A total of 516 children were immunized by 31 PHNs pre- and post-implementation in the intervention sites. Overall usage of at least one newly recommended strategy increased from 22.4% pre-intervention to 77.6% post-implementation ($p<0.01$). Post-implementation, attitudes and beliefs toward analgesic interventions were significantly ($p<0.05$) more positive. PHNs also reported significantly higher levels of confidence and satisfaction in their abilities to reduce immunization injection pain post-implementation.
Conclusion: Implementation of the new guideline improved PHN immunizers' attitudes, beliefs, and practices regarding paediatric immunization injection pain. It is anticipated that province-wide implementation of the guideline will result in a better immunization experience for children, caregivers and immunizers.

169. Acute Olanzapine Overdose in a Toddler: A Case Report with Pharmacological Insights

Tanoshima R, Chandranipapongse W, Colantonio D, Stefan C, Nulman I (The Hospital for Sick Children)

Background: Olanzapine, a dopamine and serotonin antagonist, is widely used and therefore available for accidental exposure in children. An atypical antipsychotic, olanzapine is supposed to have minimal extrapyramidal and hyperprolactinemia-related side effects.
Objectives: To define the clinical signs and pharmacokinetic properties of olanzapine in toddlers after overdose.
Methods: This report describes a 17 month-old female toddler who accidentally ingested 28 mg of olanzapine. The estimated half-life of olanzapine and its effect on prolactin secretion in toddlers is reported.
Results: The patient experienced prolonged respiratory distress, leading to four days of intubation. She also experienced fever and extrapyramidal symptoms. Olanzapine levels were measured at two different time points. At day five, her prolactin level was above the upper limits of the normal range for this age group. With supportive care, she was discharged on day seven without complications.
Conclusions: The elimination half life of olanzapine in this toddler was estimated to be 13.7 hours. This supports previous findings that the elimination half life found in toddlers is significantly shorter than in adults. This is the first case to measure prolactin levels in an olanzapine-overdosed toddler. More research is needed to elucidate the importance of this finding.

170. Transcriptional Regulation of Hepatic Drug Metabolizing Enzymes in Chronic Renal Failure Rats

Velenosi TJ, Hardy DB, Luo S, Wang H, Urquhart BL (The University of Western Ontario)

Background: Drug metabolizing enzymes such as CYP3A are highly regulated by nuclear receptors. Expression and activity of these enzymes are decreased in chronic renal failure (CRF); however the mechanism by which this is occurs is largely unknown.
Objectives: This study aimed to determine the mechanism of hepatic drug metabolizing enzyme down-regulation in CRF.
Methods: CRF in Sprague-Dawley rats was surgically induced by 5/6 nephrectomy. Control rats underwent sham laparotomies. Rats were sacrificed on day 42 and hepatic CYP3A1, CYP3A2 and CYP2C11 expression and activity were determined. Chromatin Immunoprecipitation (ChIP) was performed to determine the transcriptional activation of these enzymes by nuclear receptors.
Results: On day 42, serum creatinine levels were 23.1 ± 0.9 and 60.7 ± 10.4 μM in control and CRF rats, respectively.
Conclusions: Our results demonstrate that decreased CYP3A1/2 and CYP2C11 function and protein expression are secondary to the decrease in transcriptional activation. This study provides further insight into the mechanisms of variability in drug therapy in patients with CRF.

171. Heme Oxygenase: Selective HO-2 Inhibition

Vlahakis JZ[1], Vukomanovic D[2], Nakatsu K[2], Szarek WA[1] (Queen's University)

Background/Objective: A program in our laboratories is concerned with the design and synthesis of selective inhibitors of heme oxygenase (HO). Several azole-based compounds have been

synthesized and evaluated as novel inhibitors. Recently, a new series of benzimidazole derivatives has been synthesized and screened for HO inhibitory activity.
Methods: The compounds were tested as inhibitors of HO-1 (rat spleen microsomal fraction) and HO-2 (rat brain microsomal fraction) using an in vitro assay for heme oxygenase based on the quantitation of CO formed from the degradation of methemalbumin.
Results: Most of the compounds in this series were found to be potent inhibitors of the constitutive isozyme HO-2, showing little inhibitory activity against the stress-induced isozyme HO-1. This selectivity for HO-2 is in contrast to our previous findings from our exploration of imidazole–dioxolane derivatives, which led to the discovery of numerous potent and selective HO-1 inhibitors. The synthesis of these novel analogs and structure–activity relationships with respect to the inhibition of HO and other enzymes will be presented.
Conclusions: Our selective HO-2 inhibitors are anticipated to become useful tools in elucidating the physiological/pathological roles of heme oxygenase/carbon monoxide in mammalian and other biological systems, and complement our suite of selective HO-1 inhibitors.

172. Role of Oxidative Stress in 4-Aminobiphenyl-Induced Liver Tumorigenesis in Mice

Wang S[1], Sugamori KS[1], Grant DM[1,2] (University of Toronto)

4-Aminobiphenyl (ABP) is a probable human environmental carcinogen that also produces liver tumors in mice. Neonatal exposure of mice to ABP leads to a dramatically lower liver tumor incidence in females than males. This sex difference parallels that found in human liver cancer, where women have a three- to five-fold lower incidence than men. Given that oxidative stress represents a major etiological factor in human liver cancer, we hypothesize that sex differences in ABP-induced oxidative stress correlate with liver tumor incidence in mice. We quantified the formation of reactive oxygen species (ROS) using the dichlorofluorescein assay, lipid peroxidation by measuring thiobarbituric acid reactive substances (TBARS), and oxidative DNA damage by immunochemical detection of the H2AX protein. ABP exposure of cultured Hepa1c1c7 mouse hepatoma cells led to increased ROS formation which could be blocked by the antioxidant *N*-acetylcysteine, as well as a slight but non-significant increase in lipid peroxidation. The major oxidative ABP metabolite *N*-hydroxy-ABP also increased oxidative DNA damage in these cells. In preliminary *in vivo* studies, ABP-treated neonatal mice also showed an increase in oxidative DNA damage in liver. These results suggest that ABP is capable of producing liver oxidative stress. Ongoing studies are focused on comparing ABP-induced oxidative stress in male and female mice, and correlating these measures with liver tumor incidence. Funding for this project is received from the Canadian Institutes of Health Research and no conflict of interest is reported.

173. Determinants of CYP3A4 Expression and Metabolic Activity in the Huh7 Human Hepatoma Cell Model of Non-alcoholic Fatty Liver Disease

Woolsey SJ, Tanner J, Beaton M, Tirona RG (The University of Western Ontario)

Background: With the increased prevalence of obesity, diabetes and metabolic syndrome, Non-Alcoholic Fatty Liver Disease (NAFLD) has become the most common liver disease. While medication use is common in NAFLD to treat comorbidities, little is known regarding the effect of hepatic steatosis on drug elimination and drug response. Studies in our laboratory have recently demonstrated that Cytochrome P450 (CYP) 3A4 metabolic activity and expression are decreased in NAFLD. Here, we explored the potential molecular mechanisms that may be involved.
Objectives: To examine possible transcriptional mechanisms of CYP3A4 expression *in vitro* and *in vivo*.
Methods: Cultured Huh7 human hepatoma cells exposed with fatty acids to induce steatosis were transfected with a CYP3A4 promoter firefly luciferase reporter. C57BL/6 mice were fed a high fat diet for 5 weeks as a model of NAFLD. Hydrodynamic tail-vein injection was used to deliver the CYP3A4 luciferase reporter into the liver for functional analysis.
Results: Reporter gene analysis demonstrated that CYP3A4 transcriptional activity decreases in our *in vitro* and *in vivo* NAFLD models in comparison to control by 12% and 60% respectively.

Conclusions: We conclude that drug metabolism activity is reduced in NAFLD due to steatosis-induced reduction in CYP3A4 gene transcription. These findings are expected to provide the basis for further studies aimed at optimizing pharmacotherapy in NAFLD.

174. Characterization of Novel Pathogenic Roles of Cytochrome P450s in Endometrial Cancer Cells

Yang X, Guilarte LGM, Armstrong C, Turgeon J (University of Montreal)

Background: Endometrial cancer (EC) cells show resistance to chemotherapy agents thereby resulting in resistance and failure in cancer treatment. This phenomenon could be due to the expression of membrane transporters in cancer cells as well as due to the expression of highly active drug metabolizing enzymes. In this study, we evaluated the implication of CYP450s in the local metabolism of drugs by EC cells by evaluating their expression levels in several cell lines. The metabolic activity of microsomes prepared from these cells was evaluated as well using probe drug substrates.
Methods: Extracted mRNA from 4 EC cell lines were analyzed by RT-PCR. Microsomes were prepared from an optimized procedure and activities measured for ebastatine (CYP2J2, 1-25 μM), chlorzoxazone (CYP2E1, 600 μM), midazolam (CYP3A5, 2 μM), bupropion (CYP2B6, 310-1550 μM), ethoxyresorufin (CYP1A1 and 1B1, 1-20 μM).
Results: The expression levels of CYP450 mRNAs showed great variability. CYP1A1 and 1B1 were highly expressed in HEC 1-B, KLE, and RL-95-2 cells while little expressed in AN3CA cells where CYP 2E1, 2A6 and 2D6 were major contributors. CYP2J2 and 2B6 are also major contributors in HEC1-B cells. Other types of CYP450s showed little to no expression. In HEC 1-B cells, CYP 2J2 had higher activity than CYP 2E1 and 3A5 while CYP1A1, 1B1 and 2B6 did not show any activity.
Conclusions: CYP450 are expressed and active at various degrees in various EC cell lines.

175. Bioavailability of Amoxicilin Dissolved in Human Milk

Yazdani-Brojeni P, Garcia-Bournissen F, Fujii H, Tanoshima R, Ito S (Hospital for Sick Children)

Background: Since the safety of drinking water in some developing countries is a concern, breast milk could be an appropriate substitute of water to dissolve amoxicillin. No evidence is currently available to prove bioequivalence of drugs dissolved in breast milk compared to those dissolved in water.
Method: We conducted a randomized 2x2 cross-over single-dose PK study in healthy adult volunteers to characterize basic PK parameters of amoxicillin dissolved in human milk or water. Sixteen healthy adult volunteers (male or female) were enrolled. 500 mg amoxicillin was orally administered to each fasting volunteer (10 hrs). 8 blood samples were collected. Amoxicillin plasma concentrations were determined by HPLC-MS/MS. Amoxicillin PK parameters were estimated using a model-independent approach. Means of the bioequivalence parameters (C max and UAC) in 2 groups are compared using paired Student t-test.
Results: While the inter-individual variations of the concentration profiles were apparent, the intra (i.e., within)-individual differences between the water and milk arms were not remarkable. There was no statistically significant difference in C max and AUC between the 2 vehicle arms (i.e., water and human milk).

Registrants (as of June 3, 2012)

Abbott, Frank	Univ of British Columbia
Abdulla, Dalya	Sheridan College
Acton, Brooke	University of Toronto
Adebayo, Ganiyu	Sligo General Hospital
Adeyanju, Kaothar (Kemi)	University of W. Ontario
Aghazadeh-Habashi, Ali	University of Alberta
Akinwumi, Bolanle	University of Manitoba
Alamilla, Javier	McMaster University
Albar, Wed	University of W. Ontario
Alhowiriny, Tawfeq	King Saud University
Allen, Christine	University of Toronto
Alomrani, Abdullah	King Saudi University
Alshamsan, Aws	King Saud University
Alshememry, Abdullah	King Saud University
Alsmeyer, Daniel	Apotex
Althurwi, Hassan	University of Alberta
Amiji, Mansoor	Northeastern University
Aminkeng, Folefac	Univ of British Columbia
Amstutz, Ursula	Univ of British Columbia
Aoki, Fred	University of Manitoba
Aoun, Valery	Université de Montreal
Asghar, Muhammad Waheed	University of Alberta
Ashraf, Tamima	University of Toronto
Attafi, Ibraheem	King Saud University
Badea, Ildiko	Univ of Saskatchewan
Bai, Donglin	University of W. Ontario
Ballmer, Patrick	Sotax
Banerjee, Nilasha	University of Toronto
Barralet, Jake	McGill University
Barszczyk, Andrew	University of Toronto
Bastin, Guillaume	University of Toronto
Bayoumi, Mohsen	King Saud University
Belisle, Manon	Teva Canada
Bello, Angelica	University Health Network
Ben.Eltriki, Mohamed	University of Alberta
Bendayan, Reina	University of Toronto
Bennett, Brian	Queen
Berger, Neil	Pharma Medica Research
Bergman, Hagit	Hospital for Sick Children
Best, Arielle	Western University
Bhatia, Amit	Lovely Professional Univ
Binkhathlan, Ziyad	King Saud Univeristy
Bogh, Kelly	Cdn Science Publishers - NRC Press
Borgland, Stephanie	Univ of British Columbia
Bralha, Fernando	University of Toronto
Bratoeff, Eugene	University of Mexico City
Brien, James	Queen
Brocks, Dion	University of Alberta
Brunck, Lynne	Bayer Inc.
Burczynski, Frank	University of Manitoba
Burdo-Panasiuk, Pina	Pharma Medica Research
C.-Gaudreault, René	Université Laval
Cabeza, Marisa	Universidad Autónoma Metropolitana-Xochimilco
Calderon, David	Instit Nacional De Pediatria
Carelton, Bruce	Univ of British Columbia
Carey, Nathalie	Hospital for Sick Children
Carnevale, Amanda	Hospital for Sick Children
Cerullo, Dante	Agilent
Chamberlain, Graham	University of Toronto
Chan, Cathy	University of Alberta
Chandranipapongse, Weerawadee	Hospital for Sick Children
Chang, Thomas	Univ of British Columbia
Chattopadhyay, Niladri	University of Toronto
Chau, Kim	Hospital for Sick Children
Chaudhary, Hetal	University of Alberta
Chaudhry, Shahnaz	Hospital for Sick Children
Chefson, Amandine	Université de Montréal
Chen, Kuan	University of Toronto
Chen, Christopher	University of Alberta
Cheung, Richard	Fasken Martineau DuMoulin LLP
Cheung, Melissa	Ontario Cancer Institute
Chhatre, Vinay	Northern Lipids
Choi, Chonguk	University of Toronto
Choi, Jeehye (Linda)	Sunnybrook Research Inst
Chow, Edwin	University of Toronto
Chu, Michael	University of Toronto
Chu, Stephanie	University of Manitoba
Clarridge, Mary Jane	ESBE Scientific
Constantinides, Panayiotis	Biopharmaceutical & Drug Delivery Consulting, LLC
Cressman, Alex	CSPT
Cuello, Claudio	McGill University
Cui, Zhengrong	Univ of Texas at Austin
Cullis, Pieter	University of BC
Cummins, Carolyn	University of Toronto
D, Yohan	Queen
Dadashzadeh, Simin	Shahid Beheshti University
Daneshtalab, Noriko	Memorial Univ of Nfld.
Davidovits, Judith	Hospital for Sick Children
Davies, Neal	University of Manitoba
Davit, Barbara	US-FDA/CDER
Dedina, Liana	Hospital for Sick Children
DeGorter, Marianne	University of W. Ontario
Delano, Kaitlyn	Hospital for Sick Children
Dercho, Ryan	CDRD
DiBella, John	Simulations Plus
Dresser, George	Western University
Ducharme, Murray	Learn and Confirm Inc.
Duerksen, Heather	Univ. of Saskatchewan
Durk, Matthew R.	University of Toronto
El-Bagory, Ibrahim	King Saud University
El-Housseini, Arwa	University of Montreal
Elvin, Alfred	Anatase Biopharmaceutic Consulting, LLC
Elzagallaai, Abdelbaset	Western University
Endrenyi, Laszlo	University of Toronto
Ernsting, Mark	OICR
Etwel, Fatma	UWO
Feere, David	University of W. Ontario
Feng, Zhong-Ping	University of Toronto

Fisher, Harold	Eli Lilly Canada
Fortin, Sébastien	Univ du QC à Trois-Rivières
Fotaki, Nikoletta	University of Bath
Frank, Markus	Harvard Medical School
Gahir, Sarabjit	University of Toronto
Gareri, Joey	Hospital for Sick Children
Gee, Barry	Centre for Drug Research and Development
Gillespie, Deda	McMaster University
Gong, Yuewen	University of Manitoba
Gong, Inna	Western University
Grant, Denis	University of Toronto
Greig, Joanne	Croda Canada Ltd
Grosse, Laurent	Laval University
Grubich, Brian	OptumInsight (Canada)
Gryn, Steven	Western University
Hall, Marsha	Astellas Pharma Canada
Hammond, James	University of W. Ontario
Hanly, Lauren	University of W. Ontario
Hanna, Daniel	University of Toronto
Hewitt, Stephanie	Memorial Univ of Nfld
Heximer, Scott	University of Toronto
Ho, Emmanuel	University of Manitoba
Hoti, Alban	Etika Pharm
Huang, Leaf	UNC Chapel Hill
Huguet, Jade	University of Montreal
Hum, Maaike	Queen
Hunter, Sarah	University of Toronto
Hussain, Syed	Zef Scientific
Hussaini, Syed	Agilent
Ibrahim, Fady	Univ of British Columbia
Ito, Shinya	Hospital for Sick Children
Jamali, Fakhreddin	University of Alberta
Jandoc, Racquel	McMaster University
Jimenez, Ricardo	UBC
Jones Chukwudi, Ozokwere	Tswane Univ of Technology
Jong, Will	Hospital for Sick Children
Juurlink, David	Institute for Clinical Evaluative Sciences
Kahouli, Imen	McGill University
Kamiya, Taro	Hospital for Sick Children
Kanfer, Isadore	Rhodes University
Kapur, Bhushan	Hospital for sick Children
Karim, Sultan	Univ of British Columbia
Karimi-Busheri, Feridoun	NovaRx Corporation
Kaur, Randeep	Univ of Saskatchewan
Kelley, Shana	University of Toronto
Kelly, Lauren	University of W. Ontario
Kennedy, Deborah	Hospital for Sick Children
Kim, Richard	London Health Sciences Centre
Kis, Olena	University of Toronto
Klein, Agnes	Health Canada
Knauer, Michael	University of W. Ontario
Kolomeyets, Natalya	Zef Scientific
Konig, Rhiannon	Purdue Pharma
Korashy, Hesham M.	King Saud University
Koren, Gideon	University of Toronto, Hospital for Sick Children
Kovacs, Elisabeth	Apotex
Kowalec, Kaarina	University of BC
Kwong, Elizabeth	Kwong Eureka Solutions
Lam, Kyla	University of Toronto
Lam, Jessica	Hospital for Sick Children
Lamb, Elaine	Pfizer Consumer Healthcare
Landry, Kathryn	Dalhousie University
Leblond Chain, Jeanne	Université de Montréal
Leclair, Gregoire	Université de Montréal
Lee, D	NoAb BioDiscoveries
Lee, Sue	Center for Drug Research & Developement
Legan, Robin	London Health Sciences Centre
LePage, Robert	Pharma Medica Research
Lesko, Larry	University of Florida
Leung, Yat Hei	University of Montreal
Li, Shyh-Dar	OICR
li, jason	University of Toronto
Loebenberg, Raimar	University of Alberta
Loida, Oruña	CECMED
Lu, Jennifer	Montreal University
Lutter, Lorelei	Bio Pharma Services
Maayah, Zaid H.	King Saud University
MacKenzie, Connie	Western University
Madadi, Parvaz	Hospital for Sick Children
Magomedova, Lilia	University of Toronto
Martinez, Stephanie	University of Manitoba
Massicotte, Julie	Algorithme Pharma
Mathai, Jaigi	Health Canada
Matlow, Jeremy	Hospital for Sick Children
Matsui, Doreen	University of W. Ontario
Matsui, William	Cancer Centre at Johns Hopkins
May, Jonathan	OICR
McGilveray, Iain	McGilveray Pharmacon Inc
Mejia, Edgard	University of Manitoba
Melkoumov, Alexandre	Université de Montréal
Mielke, John	University of Waterloo
Mighiu, Alexandra	University of Toronto
Mikhail, Andrew	University of Toronto
Miller, Donald	University of Manitoba
Miller, Lutfiya	University of Toronto
Mitchell, Jane	OptumInsight
Murakami, Mami	OICR
Nash, Jordan	University of Manitoba
Natekar, Aniket	Hospital for Sick Children
Nazari, Mozhgan	University of Toronto
Neuman, Manuela	MaRS Discovery Centre
Ngo Ndjock Mbong, Ghislaine	University of Toronto
Noueihed, Baraa	McGill University
Novakovic, Jasmina	Apotex Inc
Nulman, Irena	Univeristy of Toronto
On, Ngoc	University of Manitoba
Orava, Erik	Sunnybrook Research Inst.
Ormsby, Eric	Health Canada
Oruna-Sanchez, Loida	Latin American School of Medicine
Pang, Catherine	UBC
Parikh, Chaitya	University of Toronto
Park, Jamie	University of W. Ontario
Parkinson, Fiona	University of Manitoba
Parsons, Gay	Janssen Inc.

Patel, Hiren	University of Toronto
Patel, Rucha	University of Toronto
Petrovic, Vanja	University of Toronto
Pollak, P. Timothy	University of Calgary
Pourmorad, Fereshteh	Mazandaran University of Medical Sciences
Prasad, Preethy	University of Toronto
Purohit, Meena	University Health Network
Rabanel, Jean-Michel	Université de Montréal
Rajakulendran, Nishani	University of Toronto
Ramcharran, Ram	Sotax
Rasouli-nia, Aghdass	Cross Cancer Institute
Razumienko, Eva	University of Toronto
Reiz, Joseph	Purdue Pharma
Riddick, David	University of Toronto
Rieder, Michael	University of W. Ontario
Robillard, Kevin	University of Toronto
Ross, Colin	UBC
Russell, Evan	University of W. Ontario
Sadeghian, Saeed	Tehran Univ of Medical
Safaei Nikouei, Nazila	University of Alberta
Saha, Shyamali	McGill University
Sanaee, Forughalsadat	University of Alberta
Sankar, Hari	Watson Pharmaceuticals
Sarfraz, Muhammad Khan	University of Alberta
Sayre, Casey	University of Manitoba
Seeman, Philip	University of Toronto
Senkowski, Stan	ESBE Scientific
Shahin, Mostafa	University of Alberta
Shalviri, Alireza	University of Toronto
Shamshuddin, Muffadal	Univ of British Columbia
Shan, Dan Mary	University of Toronto
Shanthakumar, Narmatha	Cobalt Pharmaceuticals Inc
Sharma, Devinder	Univ of British Columbia
Shaw, Kaitlyn	Univ of British Columbia
Shaw, Julie	University of Toronto
Shere, Mahvash	Hospital for Sick Children
Shere, Mahvash	Hospital for Sick Children
Sims, Stephen	CPS
Singh, Jagbir	Univ of Saskatchewan
Sitar, Daniel	University of Manitoba
Siu, Michelle	University of Toronto
Smart, Sarah	University of Toronto
Smith, Mary	Accucaps
Smith, Anne	Pharmaceutical Outcomes Programme
Smy, Laura	Hospital for Sick Children
Soleymani Abyaneh, Hoda	University of Alberta
Stotz, Stephanie	Children
Sugiyama, Yuichi	RIKEN Innovation Center
Sun, Zhizhi	University of Manitoba
Sun, Dajun	University of Toronto
Sunwoo, Hoon	University of Alberta
Szirtes, Julie	Apotex Inc.
Taddio, Anna	University of Toronto
Takitani, Kimitaka	Osaka Medical College
Tanielian, Lory	Gattefossé Canada Inc.
Tanoshima, Reo	Hospital for Sick Children
Tao, Bernice	Apotex
Taylor, Anna	BioPharma Services
Thompson, Michael	McMaster University
Toal, Corey	SLIDES
Tomaro-Duchesneau, Catherine	McGill University
Toth, Katalin	Université Laval
Towers, Anita	Cetero Research
Travelsi, Fethi	BioPharma Services
Tsang, Yu Chung	Apotex Inc.
Tse, Mandy	University of Alberta
Tymianski, Michael	Toronto Western Hospital
Ubhi, Bhagwant	Pfizer Consumer Healthcare
Urquhart, Brad	University of W. Ontario
Vadas, Elizabeth	InSciTech Inc.
Van Cauwenberghe, Owen	Accucaps Industries
Velenosi, Thomas	University of W. Ontario
Villano, Jonathan	Cedarlane
Vlahakis, Jason	Queen
Vonk, Alex	University of Toronto
Walliser, Matthew	Univ of Saskatchewan
Wang, Shuang	University of Toronto
Wang, Xiaotong	University of Manitoba
Warraich, Sumeeta	University of W. Ontario
Wasan, Kishor	Univ of British Columbia
Weatherley, Sharleen	McMaster University
Wells, Peter	University of Toronto
Wheal, Benjamin	University of W. Ontario
Wilson, Kaley	CDRD
Woodin, Melanie	University of Toronto
Woodland, Cindy	University of Toronto
Woolsey, Sarah	University of W. Ontario
Wu, Shirley	University of Toronto
Xiang, Fuli	Western University
Yang, Guangdong	Lakehead University
Yang, Xiaojuan	University of Montreal
Yazdani Brojeni, Parvaneh	Hospital for Sick Children
Yeghikyan, Levon	BioPharma Services Inc.
Yeung, Pollen	Dalhousie University
Young, Robert	Simon Fraser University
Zhang, Rui Xue (Vicki)	University of Toronto

Index

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Journal of Pharmacy & Pharmaceutical Sciences

A free and open access peer-reviewed online journal since 1998 - the first in the field
Published by the Canadian Society for Pharmaceutical Sciences
JPPS is cited in: Chemical Abstracts, Current Contents, Embase, Index Medicus, Pubmed
ISSN: 1482-1826; ISI Impact Factor: 2010, 1.914; 5-year, 2.517

Selected Articles from J Pharm Pharmaceut Sci (www.cspscanada.org) Vol 15, No 2 (2012):