
12th Canadian Society for Pharmaceutical Sciences (CSPS) Annual Meeting

June 3-6, 2009
The Hyatt Regency
Toronto, Ontario, Canada

International Symposium on Pharmacy & Pharmaceutical Sciences:

Drug Development to Regulatory Approval

Organizing Committee

Scientific Chairs

Laszlo Endrenyi, University of Toronto, Toronto, ON
Yu Chung Tsang, Apotex Inc., Toronto, ON

Local Organizing Committee

Rav Kumar, GlaxoSmithKline, Toronto, ON
Reina Bendayan, University of Toronto, Toronto, ON
Brian Foster, Health Canada, Ottawa, ON
Bev Incledon, Pacgen Biopharmaceuticals Corporation, Toronto, ON
Fakhreddin Jamali, University of Alberta, Edmonton, AB
Herman Lam, Calibration Validation Group (CVG), Toronto, ON
Lorelei Lutter, Bio Pharma Services Inc., Toronto, ON
Iuliana Pop, ICAR Consulting: Library and Information Services, Richmond Hill, ON
Yu Chung Tsang, Apotex Inc., Toronto, ON

Symposium 2009 Awards

Fellow Award Recipients:

Fakhreddin Jamali, Ph.D., Professor, Faculty of Pharmacy, University of Alberta, Edmonton, Alberta

Iain McGilveray, Ph.D., McGilveray Pharmacon Inc., Ottawa, Ontario

CSPS Award of Leadership in Canadian Pharmaceutical Sciences (2009):

Recipient: Helen M. Burt, Ph.D., Angiotech Professor of Drug Delivery and Associate Dean, Research and Graduate Studies, Faculty of Pharmaceutical Sciences, UBC; Division Head, Drug Delivery, Centre for Drug Research and Development, Vancouver, BC

CSPS/GlaxoSmithKline Early Career Award (2009):

Recipient: Stephane Angers, Ph.D., Assistant Professor, Faculty of Pharmacy & Department of Biochemistry, University of Toronto, Toronto, Ontario

Gattefosse Canada/CSPS Lipid-Based Drug Delivery Award (2009):

Recipient: Marie-Christine Jones, Ph.D., (Laboratory of Jean-Christophe Leroux, Faculty of Pharmacy, University of Montreal) for their manuscript entitled "*Reverse Polymeric Micelles for Pharmaceutical Applications*" published in Journal of Controlled Release 132 (2008) 208-215.

Poster Awards - Winners to be announced at the Symposium Gala Dinner:

- **Antoine A. Noujaim Award of Excellence**
- **Biovail Contract Research Award of Excellence**
- **Cedarlane Award of Excellence**

National Summer Student Research Program Awards sponsored by Merck-Frosst Canada Ltd. (2009):

Anthony Gador, University of British Columbia (Supervisor: Dr. Kathleen McLeod)

Topic: Role of iNOS and Reactive Oxygen Species in Activation of RhoA/Rho Kinase Pathway in Diabetic Cardiomyopathy

Michael Prout, University of Manitoba, (Supervisor: Dr. Mike Namaka)

Topic: An Interim Analysis: The Role of Superantigen Producing Staphylococcus aureus in the Etiology of Multiple Sclerosis

Trevor Elton, University of Alberta (Supervisors: Dr. Mark Makowsky & Dr. Ross Tsuyuki)

Topic: Differences in Prevalence and Treatment Patterns of Lower Extremity Peripheral Arterial Disease in Urban and Rural Communities in Alberta (epiPAD)

Thomas Veinot, Dalhousie University (Supervisor: Dr. David Jakeman)

Topic: Structural Elucidation of Drug Metabolites and Identifying New Antibiotics Using Mass Spectrometry Unit

Jesse Goldmacher, University of Toronto (Supervisor: Dr. Carolyn Cummins)

Topic: Investigating Insulin and Glucocorticoid Signaling in LXR-Deficient Mice

Joshua Buse, University of Saskatchewan (Supervisor: Dr. Anas El-Aneed)

Topic: Similarities and Differences in the Mass Spectrometric Fragmentation Patterns of a Series of Novel Gemini Surfactants Used in Non-Viral Gene Delivery

About CSPS

The Canadian Society for Pharmaceutical Sciences (CSPS), is a non-profit organization which was 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.

Society Mission

CSPS is the premier organization for bringing together pharmaceutical scientists in Canada to advance excellence in Canadian pharmaceutical R&D and education.

Our Vision

To bring together researchers in academia, industry, and government, and advance pharmaceutical sciences, drug discovery and development in Canada.

Strategic Objectives

- Achieve long term sustainability through a solid funding and operation model.
- Maximize the potential of the *Journal of Pharmacy and Pharmaceutical Sciences*.
- Enhance and grow the Annual Symposium - the premiere meeting for Canadian pharmaceutical sciences.
- Build partnerships and develop a strong voice to encourage government, academia, and industry to advance pharmaceutical R&D innovation in Canada.

Executive Council

- *Rav Kumar* (President), GlaxoSmithKline, Mississauga, Ontario
- *Laszlo Endrenyi* (President-Elect), University of Toronto, Toronto, Ontario
- *Leanne Embree* (Past President), LinkCore Pharma Corporation, Vancouver, British Columbia
- *Lorelei Lutter* (Secretary), Bio Pharma Services Inc., Toronto, Ontario
- *Brian Foster* (Treasurer), Health Canada, Ottawa, Ontario
- *Fakhreddin Jamali* (Appointed By Executive Council), University of Alberta, Edmonton, Alberta
- *Frank Burczynski* (Member-At-Large), University of Manitoba, Winnipeg, Manitoba
- *Kishor Wasan* (Member-At-Large), University of British Columbia, Vancouver, BC
- *Isadore Kanfer* (Member-At-Large), University of Toronto, Toronto, Ontario
- *Yu Chung Tsang* (Member-At-Large), Apotex Inc., Toronto, Ontario
- *Reina Bendayan* (Member-At-Large), University of Toronto, Toronto, Ontario
- *Daryl Fediuk* (Trainee Representative), University of Manitoba, Winnipeg, Manitoba

CSPS Contact Information

Canadian Society for Pharmaceutical Sciences
3126 Dentistry/Pharmacy Centre
University of Alberta Campus
Edmonton, Alberta, Canada, T6G 2N8

Telephone: 780-492-0950
Fax: 780-492-0951
Email: bberekoff@cspscanada.org

Executive Director: Barbara Scollick
Administrator: Bev Berekoff

Journal of Pharmacy & Pharmaceutical Sciences

A free and open access peer-reviewed online journal since 1998 - the first in the field
JPPS is cited in: Chemical Abstracts, Current Contents, Embase, Index Medicus, Pubmed
ISI Impact Factor: 5-Year, 2.24; 2007, 1.7

Selected articles from J Pharm Pharmaceut Sci (www.cspscanada.org) 12(1) 2009:

Review Articles

Emerging Significance of Flavonoids as P-Glycoprotein Inhibitors in Cancer Chemotherapy

Tripta Bansal, Manu Jaggi, Roop Khar, Sushama Talegaonkar, India

The Use of Sonophoresis in the Administration of Drugs Throughout the Skin

Jose Juan Escobar-Chavez, Dalia Bonilla-Martínez, Martha Angélica Villegas-González, Isabel Marlen Rodríguez-Cruz, Clara Luisa Domínguez-Delgado, Mexico

Original Articles

Pharmacokinetic Interaction Between Oltipraz and Silymarin in Rats

Min Kyung Kang, Soo Kyung Bae, Jin Wan Kim, Myung Gull Lee, Republic Of Korea

Rheological Investigation of Self-emulsification Process

Shailesh V Biradar, Ravindra S Dhumal, Anant Paradkar, India

Predictors of Pharmacy Students' Intentions to Monitor Diabetes

Lisa M Guirguis, Betty A Chewning, Mara A Kieser, Canada, United States

Effects of Rivastigmine and Donepezil on Brain Acetylcholine Levels in Acetylcholinesterase-Deficient Mice

Runa S. Naik, Joachim Hartmann, Cornelia Kiewert, Ellen G. Duysen, Oksana Lockridge, Jochen Klein, United States, Germany

Monoterpenoids Induce Agonist-Specific Desensitization of Transient Receptor Potential Vanilloid-3 (TRPV3) ion Channels

Muhammad Azhar Sherkheli, Heike Benecke, Julia Franca Doerner, Olaf Kletke, A. K. Vogt-Eisele, Guenter Gisselmann, Hanns Hatt, Germany

Design and Evaluation of a Novel Floating Osmotic Pump System

Zhihong Zhang, Bo Peng, Xinggong Yang, Chao Wang, Guangmei Sun, Weisan Pan, China

Notes

No Decrease in Central Venous Pressure when Epinephrine-Induced Hypotension Occurs

Jian-jun Yang, Xiao-li Xu, Jin-chun Shen, Wei-yan Li, Zhi-qiang Zhou, China

Conference Program

Drug Development to Regulatory Approval

Wednesday, June 3, 2009

CSPS WORKSHOP:

9:30 AM – 5:00 PM **Evaluation of In Vitro-In Vivo Correlation (IVIVC)**, presented by Jason Chittenden, Pharsight Corporation

EXCIPIENT WORKSHOP:

10:00 AM – 5:00 PM A technical forum aimed at the Canadian Pharma Formulation community. Presentations from six global excipient suppliers on new products, studies, and formulation technologies.

3:00-7:00 PM **Registration**

5:30-7:00 PM **Wine and Cheese Reception** (complimentary)

Thursday, June 4, 2009

7:30 AM **Registration & Breakfast**

8:00 AM - 5:00 PM **Poster Presentations**

8:30 PLENARY SESSION (SPONSORED BY FMC BIOPOLYMER)

8:30 **Welcome** - Dr. Rav Kumar, CSPS President

8:40 **Honourable John Wilkinson**, Minister of Research and Innovation, Government of Ontario: "Importance of Pharmaceutical Research in Canada"

9:10 **Gordon Amidon**, University of Michigan, Ann Arbor, MI
"Biopharmaceutics and the Renaissance of an Old Science: In Vitro-In Vivo Dissolution"

10:00 Poster Viewing and Break

Award Winner Presentations:

10:30 **GlaxoSmithKline Early Career Award** - **Dr. Stephane Angers**, University of Toronto
"Functional Proteomics of G Protein Coupled Receptors Signalling"

11:00 **CSPS Leadership Award** - **Dr. Helen Burt**, University of British Columbia, Vancouver, BC
"Polymeric Drug Delivery Systems: Size Matters!"

11:30 Luncheon and CSPS Annual General Meeting

	Thursday, June 4, 2009 (cont'd)	
1:30	Session I a	Session I b
	DRUG THERAPY AND MEMBRANE TRANSPORTERS: CLINICAL, INDUSTRIAL AND GOVERNMENTAL ASPECTS Chairs: Reina Bendayan, University of Toronto; Toronto, ON; Brian Foster, Health Canada, Ottawa, ON	DEVELOPMENT OF BIOPHARMACEUTICALS Chairs: Leanne Embree, LinkCore Pharma, Vancouver, BC; Bev Incledon, Pacgen Biopharmaceuticals, Toronto, ON
1:30	Clinical Significance of Drug Efflux Transporters Frances Sharom, University of Guelph, Guelph, ON	Biomarkers and Drug Development Terrence Sills, Ontario Biomarker Network, Toronto, ON
2:00	The Use of Lipid Excipients to Circumvent Multidrug Resistance Kishor Wasan, University of British Columbia, Vancouver, BC	Dirucotide: A Therapy for Multiple Sclerosis Randy Stroud, BioMS Technology, Scarborough, ON
2:30	Poster viewing and break	
3:00	Drug Transporters and Potential for Drug-Drug Interactions Raymond Evers, Merck USA, Rahway, NJ	Issues to Consider During the Development of a Biotechnology Product Bruce Meiklejohn, Eli Lilly and Co., Indianapolis, IN
3:30	Interactions of Nutraceuticals and Natural Compounds with Membrane Transporters and Metabolic Enzymes: Government Perspective Brian Foster, Health Canada, Ottawa, ON	Pharmaceutical Development and Lead Candidate Selection Ron Boch, Pharmaceutical Consulting, North Vancouver, BC
4:00	Panel discussion	Panel discussion
4:30	Poster Viewing	

Friday, June 5, 2009		
7:00 - 8:20 AM Sunrise Breakfast Session for Trainees (anyone welcome)		
	7:00 Introduction to Pharmacokinetic (PK) Models & Data Analysis Laszlo Endrenyi, University of Toronto	
	7:35 Illustration of PK Models & Analysis of Actual Data by WinNonlin Jason Chittenden, Pharsight Corporation	
7:30 AM Breakfast		
8:00 AM Registration		
8:00 AM - 5:00 PM Poster Presentations		
8:30	Session II a	Session II b (SPONSORED BY McDOUGALL SCIENTIFIC)
	INTEGRATION OF IMAGING IN PHARMACEUTICAL RESEARCH AND DEVELOPMENT Chair: Christine Allen, University of Toronto, Toronto, ON	ASSESSMENT OF BIOEQUIVALENCE FOR DRUGS REQUIRING PHARMACODYNAMIC MEASURES OR CLINICAL ENDPOINTS Chairs: Isadore Kanfer, Rhodes University, Grahamstown, South Africa; Lorelei Lutter, BioPharma Services, Toronto, ON
8:30	The Pharmaceutical Industry's Perspective on Imaging in Drug Development Raymond Gibson, Merck/MSDRL, Holland, PA	Bioequivalence Assessment of Topical Dermatological Dosage Forms: "Official" and Investigative Methods Isadore Kanfer, Rhodes University, Grahamstown, South Africa

	Friday, June 5, 2009 (cont'd)	
9:00	In Vivo Molecular Imaging of Oligonucleotides Bertrand Tavitian, INSERM (Institut National de la Sante et la Recherche Medicale), Orsay, France	Bioequivalence of Dosage Forms Administered Orally but Not Intended for the Systemic Circulation Keith Gallicano, Watson Pharmaceuticals, Corona, CA, USA
9:30	Poster Viewing and Break	
10:00	Role of Molecular Imaging in Evaluating Tumor Response Norbert Avril, Barts and The London School of Medicine, London, UK	Inhaled Corticosteroids Murray Ducharme, Cetero Research, Cary, NC
10:30	SPECT Imaging of Tc-99m Radio-labelled Polymers Urs Hafeli, University of British Columbia, Vancouver, BC	Clinical Endpoint Studies To Include Ophthalmics, Dermatologicals and Any Other Such Products Grier Harris, Appian International, Charlotte, NC
11:00	Panel discussion	Panel discussion
11:30	Lunch & Poster viewing	
1:00	Session III a	Session III b
	INTERSUBJECT VARIABILITY IN DRUG DESIGN AND PHARMACOTHERAPY Chair: Fakhreddin Jamali, University of Alberta, Edmonton, AB Co-Chair: Amin Rostami-Hodjegan, University of Sheffield, U.K.	TRANSDERMAL DRUG DEVELOPMENT Chair: Frank Burczynski, University of Manitoba, Winnipeg, MB Co-Chair: Michael Roberts, University of Queensland, Brisbane, Australia
1:00	Rich Versus Sparse Sampling of Patient's Adherence to Pharmaceutical Product Candidates: What is Needed for Optimal Drug Development? Bernard Vrijens, University of Liège, Belgium	Recent Developments in Transdermal Drug Delivery Michael Roberts, University of Queensland, Brisbane, Australia
1:30	Disease-Drug Interaction, a Source of Intersubject Variability and Therapeutic Failure Fakhreddin Jamali, University of Alberta, Edmonton, AB	The Design of a Skeletal Muscle Regenerator Molecule that is Applied Transdermally Judy Anderson, University of Manitoba, Winnipeg, MB
2:00	A "Bottom-Up" Approach to Predict Inter-individual Variability in ADME Amin Rostami-Hodjegan, University of Sheffield, UK	Transdermal Aspects of Concurrent Use of Insect Repellent and Sunscreen Preparations: An Update Xiaochen Gu, University of Manitoba, Winnipeg, MB
2:30	Poster viewing & Break	
3:00	Session IV a	Session IV b
	INNOVATIVE DEVELOPMENT OF DRUG DELIVERY Chair: Kwok Chow, Patheon Inc., Mississauga, ON	PAT/QbD – ARE WE READY? Organizer: Herman Lam, Calibration Validation Group (CVG), Toronto, ON Chair: Bev Incledon, Pacgen Biopharmaceuticals, Toronto, ON
3:00	Development of a New Breath-Activated Powder Inhaler to Deliver Pure Drug and Higher Respiratory Fraction Jesse Zhu, University of Western Ontario, London, ON	Pharmaceutical Development - Building Robust Product Krishnan Tirunellai, Health Canada, Ottawa, ON

	Friday, June 5, 2009 (cont'd)	
3:30	Overview of Gastric Retentive Technologies to Provide Once-a-Day Dosing for Drugs with a Narrow Absorption Window Eddie Hou and Verne Cowles, Depomed Inc., Menlo Park, CA	Industrial Perspectives of PAT Ricardo Vargas, Purdue Pharma, Pickering, ON
4:00	Sono-Crystallization, Particle Engineering, and Improved Performance of Inhalable Pharmaceutical Particles Graham Ruecroft, Prosonix Ltd., Oxford, UK	Recent Advances in the Application of FT-NIR for PAT Chris Heil, Thermo Fisher Scientific, Madison, WI
4:30	Poster Viewing	
6:00	Pre-Gala Dinner Cocktails & Mixer	
7:00	CSPS GALA AWARDS DINNER (SPONSORED BY ELI LILLY CANADA)	

Saturday, June 6, 2009		
7:30 AM	Breakfast	
9:00	Session V	
	CURRENT REGULATORY ISSUES ON THE DETERMINATION OF BIOEQUIVALENCE Chairs: Laszlo Endrenyi, University of Toronto, Toronto, ON; Yu Chung Tsang, Apotex Inc., Toronto, ON	
9:00	Bioequivalence of Complex Drug Products: Regulatory Science and Its Implications for Patient Care Bruce Clark, Apotex Inc., Toronto, ON	
9:30	Implementation of the BCS Guidance: The US FDA Experience Mehul Mehta, FDA, Silver Spring, MD	
10:00	Break	
10:20	Evaluating Bioequivalence for Difficult Drug Products Barbara Davit, FDA, Rockville, MD	
10:50	Updated Guidance for the Conduct and Analysis of Bioequivalence Studies Eric Ormsby, Health Canada, Ottawa, ON	
11:20	Roundtable Discussion	
11:50	Symposium Concluding Remarks: Laszlo Endrenyi	
12:00	Symposium Adjourned	

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Speaker Abstracts

Plenary Session

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Biopharmaceutics and the Renaissance of an Old Science: *In Vitro-In Vivo* Dissolution

Gordon Amidon, College of Pharmacy, University of Michigan, Ann Arbor, MI, USA

The Biopharmaceutics Classification System, BCS, has had a significant impact on drug bioequivalence (BE) regulatory standards by bringing the focus of BE standards back to the critical processes controlling (*in vivo*) BE, Solubility-Dissolution and Permeability. While *in vivo* matching of plasma levels is the 'gold standard', insuring equivalence of plasma levels between two products containing the same drug is a scientific question of dissolution, *in vivo* dissolution. The key scientific question in setting science based regulatory standards is: What is the best dissolution test?

In order for dissolution to be most relevant to BE or a useful drug product development tool, we have to consider *in vivo* dissolution conditions which include the effects of fluid velocity (variable hydrodynamics) as well as solubilization via surfactants and lipids and pH, buffer type (pKa) and buffer capacity. These factors have only recently received the attention they require. In our work, studies of the non ionizable drug fenofibrate show the effect of both particle size and fluid velocity on dissolution can be combined with the dimensionless Reynolds number, $(d\omega/v)^{1/2}$ and is a step toward accounting for the range of hydrodynamic conditions a dosage and drug form will encounter *in vivo*. Studies with the physiological buffer bicarbonate, demonstrate significant differences from the compendia phosphate buffers. However, the effect of bicarbonate buffer can be mimicked with phosphate buffer with levels (buffer capacity) dependant on the drug solubility and pKa (Figure 3).

This presentation will summarize our most recent results on dissolution and the advances that are being made in the biopharmaceutical sciences that are making dissolution both more relevant and more important as a drug product development tool and an international regulatory standard.

Functional Proteomics of G Protein Coupled Receptors Signalling

Stephane Angers, Faculty of Pharmacy & Dept of Biochemistry University of Toronto, Toronto, ON.

Heterotrimeric G proteins are activated soon after the recognition of a wide spectrum of ligands by cell surface G protein coupled receptors. Whereas several effectors have been identified and characterized for G α subunits, less is known about how the G $\beta\gamma$ subunits exert their various biological effects. Using tandem affinity purification we have isolated G β and G γ containing protein complexes from mammalian cells and analyzed their composition by mass spectrometry. In addition to several G α subunits, the protein RADIL was identified in both G β and G γ complexes. The reciprocal isolation and analysis of RADIL complexes identified several G β and G γ proteins; validating RADIL as a novel G $\beta\gamma$ associated protein. We also found that RADIL interacts with the small GTP binding protein Rap. Given that RADIL is homologous to the adhesion protein AF6 and Rap is known to mediate inside-out signalling leading to Integrin activation, we investigated whether G $\beta\gamma$ could form a complex with RADIL and Rap1 to regulate cellular adhesion downstream of G protein coupled receptors activation. The adhesion of platelets and leukocytes during injury, the migration of cells during development, and the dissemination of cancer cells during metastasis are processes regulated by GPCRs and where the G $\beta\gamma$ -RADIL-Rap1 protein complex could be implicated.

Polymeric Drug Delivery Systems: Size Matters!

Helen M. Burt, Faculty of Pharmaceutical Sciences, Centre for Drug Research and Development, University of British Columbia, Vancouver, BC, Canada

The focus of my group's research for the past 16 years has been the design, characterization and evaluation of drug loaded polymer-based, controlled release delivery systems for a broad range of disease applications, including restenosis, cancer, arthritis and bone regeneration. These delivery systems have spanned the entire spectrum of size from nano- (micelles, hyperbranched polyglycerols, gold nanoshells) to micro- (microspheres) to macro- (stent coatings, films, implants, scaffolds). An overview of our contributions to the field will be provided.

Session I a

Drug Therapy and Membrane Transporters: Clinical, Industrial and Governmental Aspects

Clinical Significance of Drug Efflux Transporters

Frances J. Sharom, Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON

Three ATP-binding cassette (ABC) superfamily drug transporters, P-glycoprotein (ABCB1), BCRP (ABCG2), and MRP1 (ABCC1) play an important role in normal physiology. These efflux pumps protect normal tissues from both toxic endogenous metabolites and xenobiotics. They display overlapping substrate specificity, and affect the uptake and distribution of many clinically important drugs. The presence of P-glycoprotein and ABCG2 in intestinal epithelial cells, and in the endothelial cells of the blood brain barrier, limits oral bioavailability and brain penetration of many drugs used in pharmacotherapy.

Drug transporters are also expressed in many human tumours, and likely make a major contribution to resistance to chemotherapeutic drug treatment. Many chemical modulators are known to block the transport activity of P-glycoprotein, while fewer have been identified for ABCG2 and MRP1. Although the expression of these transporters in human tumours has been linked to poor prognosis, attempts to inhibit their activity using chemical modulators have led to disappointing results in clinical trials. However, the use of modulators to enhance oral drug bioavailability and delivery to the brain may improve the future therapeutic success of many drugs.

P-glycoprotein and the ABCG2 homodimer comprise two cytoplasmic nucleotide-binding domains (NBDs) and two transmembrane domains (TMDs), whereas MRP1 possesses an additional N-terminal TMD of unknown function. ABC drug efflux transporters contain a flexible substrate binding pocket, which can accommodate many amphipathic drugs of diverse chemical structure. The recent high resolution X-ray crystal structures of

P-glycoprotein bound to stereoisomers of a cyclic peptide inhibitor have revealed the principles of multidrug binding. Each drug makes numerous contacts with a different subset of aromatic and hydrophobic residues inside a cavity that is large enough to accommodate two drug molecules simultaneously. Drugs enter the cavity, which is contained within the cytoplasmic leaflet of the TMDs, after partitioning into the lipid bilayer. Transport is driven by hydrolysis of ATP, which promotes tight association of the NBDs to form a nucleotide sandwich dimer. Drugs may be expelled into the extracellular aqueous phase (hydrophobic vacuum cleaner), or into the membrane outer leaflet (drug flippase). Positive and negative effects on transport observed between pairs of drugs *in vitro* may give rise to complicated drug interactions *in vivo*.

Polymorphisms of ABC drug transporters may be responsible for varying responses to drug therapy, and have become of increasing interest. However, data has been inconclusive overall, and the impact of genotype on the expression and function of these drug transporters remains controversial.

The Use of Lipid Excipients to Circumvent Multidrug Resistance

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

The classic definition of an excipient adopted by most pharmaceutical scientists is an inert substance used as a carrier, dilute and or vehicle for the **active ingredients** of a drug product. Recent literature suggests that lipid excipients and formulations incorporating these excipients with active drugs may serve as a useful approach to potentially inhibit P-gp and drug metabolizing enzymes and improve the oral bioavailability of drugs with P-gp and drug

metabolizing enzyme limited absorption. Regardless, clinically relevant doses of these potential excipient inhibitors and optimum duration of inhibition need to be considered. There are additional needs in the case of excipient inhibitors which include better understanding of the inhibition mechanism(s) and the structure-activity relationships between these excipients and their interaction with drug-efflux transporters and drug metabolizing enzymes. This presentation will attempt to help Pharmaceutical Scientists become aware of the potential activity these excipients may have on biologically processes within the body and treat them as such.

Drug Transporters and Potential for Drug-Drug Interactions

Raymond Evers, DMPK, In Vitro Technologies, Merck & Co, Rahway, NJ, USA

A wide variety of transporters have been identified, which can contribute to the absorption, distribution and elimination of drug candidates. Most drug transporters are expressed in multiple organs and show overlap in substrate specificity. In the body, cell monolayers form permeability barriers, determining transport of drugs from one tissue compartment to another. Transporters can be involved in both uptake and efflux from cells and therefore can affect the distribution of drugs into target organs and cells. Co-administration of substrates or inhibitors of transporters could potentially result in drug-drug interactions. It therefore has become of increasing importance, both from a drug discovery and development perspective, to develop *in vitro* assay systems which allow the prediction of drug candidates to cause transporter-mediated interactions. We therefore have established a range of *in vitro* assay systems for the major human drug uptake and efflux transporters of the SLC, SLCO and ABC-transporter families expressed in the liver, kidney, intestine and blood-brain barrier. Examples will be presented how these systems can be used to elucidate the molecular mechanisms by which drugs are excreted from the body, and how this information can be used to assess DDI potential.

In addition, we have initiated an effort to establish mice lacking drug transporters. In my presentation, I will discuss the importance of these mice to establish the relevance of *in vitro* transporter data and how they can be used to explain PK issues in drug discovery.

Interactions of Nutraceuticals and Natural Compounds with Membrane Transporters and Metabolic Enzymes: Government Perspective

Brian C. Foster, Therapeutic Products Directorate, Health Canada, Ottawa, and Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

Health Canada subscribes to the position that an adequate pre-marketing investigation of the safety and efficacy of a new pharmaceutical or therapeutic drug or biological (referred to here as a drug) should include characterization of its metabolism and exploration of its interactions with other substances that may affect the safety and efficacy of a product. A nutraceutical is a product that has been isolated or purified from foods and is generally sold in medicinal forms not usually associated with food. Nutraceuticals (hereafter also referred to as an NHP) are encompassed within the NHP regulations which include homeopathic preparations, substances used in traditional medicine, a mineral or trace element, a vitamin, an amino acid, an essential fatty acid, or other botanical-, animal-, or micro-organism-derived substance. From a theoretical perspective, all NHPs have the potential to interact with drugs.

Interactions with NHPs are complex and in many instances difficult to ascribe to the product. Each manufacturing process and formulation may affect the chemical composition of each product and hence the potential for interaction. NHPs may enhance the known adverse events of a drug after one or many doses. In addition, NHPs can demonstrate toxicity that are pharmacologically predictable, and dose dependent. Idiosyncratic toxicity is also possible with reactions which cannot be predicted on the basis of pharmacological properties, and are generally not dose dependent but often serious and fatal. The PD active substance or substances may not be the same substance or substances which can affect PK or disposition; they are effector compounds but with different activities. There may be competing antagonistic and synergistic interactions occurring simultaneously within the NHP. PK interactions can occur as a result of changes in activity of the drug-metabolism enzymes and transport proteins, especially cytochrome P450 (CYP450) isozyme-mediated metabolism and P-glycoprotein (Pgp)-mediated transport. Although these are the major enzymes and transporters involved, there are many other Phase I and II enzymes, and transporters that should be considered in the determination of risk interactions.

Session I b

Development of Biopharmaceuticals

Biomarkers and Drug Development

Terrence L. Sills, Ontario Cancer Biomarker Network, MaRS Centre, Toronto, ON, Canada

Biomarkers hold significant promise in changing the way we diagnose disease, the way we monitor patient progress, and the way we develop and deliver effective therapies. Biomarkers can be applied in a number of ways to enable more refined diagnoses, to determine prognosis, and to stratify patients in order to effectively tailor treatment (e.g., distinguish responders from non-responders). The ultimate aim, and utility, of biomarkers is to enable the delivery of the right treatment to the right patient at the right time. However, at the present time, there are not many successful examples of biomarkers being applied clinically to enable more effective treatment and management of disease.

The challenge to drug developers is to develop methods to stratify patients according to their propensity for drug response within the timelines imposed by the clinical development of a NCE. If such methods can be developed they can improve the chances of success for drug development programs. Biomarkers represent an obvious approach to stratifying patients. Although application of biomarkers in drug development is a growing trend, the integration of biomarker R&D into clinical drug development is not yet routinely done in the pharmaceutical industry. This is in large part due to the fact that biomarker discovery approaches applied to date do not fit the timelines of drug development, but also because the field is new and can be associated with variable outcomes. Consequently there is an urgent need to develop rapid, reliable methods for identifying biomarkers that can be used to stratify patients.

To address these needs we propose a "biomarker battery" approach whereby discovery is targeted to hundreds of putative markers that have already been identified in the literature and in association with disease processes, affected pathways, or the mechanism of action of the NCE. Identification of a classifier set of markers is done in a multiplexed fashion to enable adherence to the imposed timelines

of the clinical development of the NCE. Verification of the classifier set is then rapidly carried out using a test sample. Successful application of this approach will dramatically improve patient selection criteria for pivotal efficacy trials such that treatment effects of a novel therapeutic agent can be more accurately assessed, and new drugs given a better chance to demonstrate their benefits.

Dirucotide: A Therapy for Multiple Sclerosis

Randy Stroud, BioMS Medical Corp., Edmonton, AB, Canada

Dirucotide (MBP8298) a 17 amino acid synthetic peptide with a sequence identical to a portion of human Myelin Basic Protein (MBP) is a novel antigen-based therapy being developed for the treatment of patients with Multiple Sclerosis (MS). Antigen-based approaches for the treatment of autoimmune diseases are particularly attractive because modifications of essential immunological activities unrelated to the disease can be avoided. The concept for this antigen-based therapy came from research conducted at the University of Alberta, where the researchers developed the drug through Phase 2 clinical trials. Evaluation of the outcome of the research and the clinical trials was positive. Transitioning from a research project that included investigator-sponsored clinical trials to a global drug development programme meeting international regulatory requirements was undertaken by BioMS.

The company BioMS functions through a small team that includes individuals who have knowledge and experience in the key drug development areas. BioMS undertook management of development of a variety of areas, including CMC, preclinical, clinical and regulatory, contracting with capable vendors to progress the development programme in compliance with international standards. The development of dirucotide for patients with Secondary Progressive MS (SPMS) is advanced, at Phase 3 clinical trials, with the first pivotal confirmatory Phase 3 trial to be completed in 2009. Patients with SPMS currently

have few therapeutic choices, so the innovative new drug dirucotide represents a step forward for these patients. Chemistry, manufacturing, and controls are well developed. The preclinical development strategy was driven by attributes of dirucotide, and the completed preclinical studies indicate that dirucotide is not genotoxic, immunotoxic, embryotoxic or teratogenic, and no major target organs of toxicity were identified in the study animals. Clinical studies have provided safety data in patients that supports the development of the innovative new drug.

In 2007, BioMS entered into a partnership agreement with Eli Lilly and Company, with Lilly to further develop the drug for marketing authorizations and commercial sale globally. Drug development activities are continuing, working towards regulatory approvals over the next few years.

Issues to Consider During the Development of a Biotechnology Product

Bruce Meiklejohn, Eli Lilly and Co., Indianapolis, IN

[No Abstract]

Pharmaceutical Development and Lead Candidate Selection

Ron Boch, Pharmaceutical Consulting, North Vancouver, BC, Canada

The selection of a lead candidate to advance into development requires a multi-disciplinary approach, with input from various pharmaceutical groups, including biology, toxicology, formulation, clinical, manufacturing and regulatory. During lead product selection and optimization, a molecule's physicochemical and biological properties together with some of the typical challenges encountered in the later stages of development are considered. Unstable or poorly soluble drug molecules can require complex formulations to improve poor or variable delivery leading to higher production costs. A wide range of dosing concentrations and different formulations used in toxicology testing may present additional challenges in the design of a suitable product that is representative of the commercial dosage form. If a change in the formulation at a later stage is required, this can impact drug delivery and present development set backs. A discussion about lead candidate selection, formulation development and the final product considerations is presented.

Sunrise Session

a. Introduction to Pharmacokinetic (PK) Models & Data Analysis

b. Illustration of PK Models & Analysis of Actual Data by WinNonlin

Introduction to Models and Data Analysis in Pharmacokinetics

Laszlo Endrenyi (University of Toronto) and Jason Chittenden (Pharsight Corporation)

Various kinds of pharmacokinetic (PK) models will be introduced including those of compartmental,

physiological and population PK. Fundamentals of kinetic data analysis will be discussed. This will be followed by demonstrations of models and analyses of actual data sets, using WinNonlin.

Session II a

Integration of Imaging in Pharmaceutical Research and Development

The Pharmaceutical Industry's Perspective on Imaging in Drug Development

Raymond Gibson, Department of Imaging Research, Merck and Co. Inc., West Point, PA, USA

With the desire to develop novel drugs faster and more cost-effectively, drug companies are searching for clear strategies to manage the complex drug discovery process in terms of balancing costs, time, product value and possibility of success. To this end positron emission tomography (PET) is being utilized to aid in optimizing the decision making process. PET is a non-invasive imaging technique that provides a means to obtain information on drug effects and/or behavior during development (i.e. radiotracer delivery, occupancy etc). Novelty is paramount for drug development in the pharmaceutical industry, thus potential block-buster drugs are seldom second generation versions of previously existing drugs (though notable exceptions exist). New targets are therefore being tested as a part of novel approaches to treatment of diseases and disorders which are either poorly treated by existing drugs, or where treatments are accompanied by significant side-effects. A key feature of drug development is thus hypothesis testing: at an appropriate dose, does the putative drug provide the desired therapeutic effect? This dose may be predicted by determining the maximum dose that can be given without side-effects, or estimated from animal studies where target-site occupancy is determined via post-mortem sampling. A more satisfying approach is to determine the fractional site occupancy, as a function of plasma concentration of drug, via blockade of *in vivo* radiotracer binding. Occasionally, the drug candidate can be radiolabeled, but most drugs do not make good radiotracers for non-invasive imaging. Thus, a development program for radiotracers which occurs parallel with that of the new drug is required.

Radiotracer validation begins pre-clinically. Whilst negative results in pre-clinical studies may not alter the clinical program, they likely point to potential problems which can be focused upon

during the early clinical trials resulting in savings in preventing studies in which either required occupancy levels are not achieved, or site-specific occupancy exhibits unfavorable kinetics. Once the occupancy/kinetics of the successful first generation drug have been characterized via imaging, imaging can be used to demonstrate that a second generation drug exhibits occupancy and kinetics which are similar to or better than the primary drug.

The Imaging Department at Merck currently has the capability of utilizing a variety of imaging modalities to facilitate drug discover and development: PET and microPET, SPECT and microSPECT, MRI/MRS, HiRes Ultrasound and CT. The presentation will focus primarily on the uses of PET in drug research and development in both preclinical and clinical studies and examined some of the principal issues that PET is being used to address in terms of developing novel drugs.

In Vivo Molecular Imaging of Oligonucleotides

Bertrand Tavitian, Laboratoire d'Imagerie moléculaire expérimentale, CEA-Inserm, Orsay, France

Molecular Imaging can assess gene expression non-invasively, repeatedly and quantitatively in living subjects. Pharmaco-Imaging of oligonucleotides allows quantifying in 3-D and in the whole bodies of animals and Humans the bio-distribution time course of antisense, aptamers, interfering RNAs, ribozymes, etc. The methodology and examples of applications for the assessment of targeting and delivery of oligonucleotides will be presented.

Use of oligonucleotides as diagnostic contrast agents is a longer way ahead of us and remains still an open question. It will require that a sufficient contrast is obtained *in vivo* and a correlation between tracer and target concentrations, two issues that are yet to be demonstrated.

Role of Molecular Imaging in Evaluating Tumor Response

Norbert Avril, Barts and The London School of Medicine, Queen Mary College, University of London, UK

Positron emission tomography (PET) uses radiolabelled molecules for visualization, characterization and quantification of biological processes such as tumor glucose metabolism and cell proliferation. Glucose metabolism is often up-regulated in malignant tumors resulting in increased cellular uptake of the glucose analogue F-18 fluorodeoxyglucose (FDG).

Prediction of treatment response refers to early identification of treatment effectiveness by comparing the level of radiotracer uptake, which reflects tumor glucose metabolism, before and after one (or more) cycle(s) of systemic therapy. The concept of using FDG-PET for monitoring therapeutic response is based on changes in tumor glucose utilization and the close correlation of early changes in tumor FDG uptake with the effectiveness of treatment. Generally, the tumor FDG uptake is assessed at baseline prior to treatment and relative changes in FDG uptake (SUV) are often used to define metabolic response. Depending on the treatment regimen and the timing of FDG-PET, various thresholds for relative decreases in SUV were found in metabolic responders and non-responders.

Several studies confirm a recently identified characteristic behavior of malignant tumors, namely the close correlation between the early decrease in glucose metabolism measured by FDG-PET and treatment response. These findings establish the basis for the future clinical application of sequential FDG-PET imaging as *in vivo* test for chemosensitivity. Shrinkage and resolution of a tumor mass is the final step in a complex cascade of cellular and sub-cellular changes induced by treatment. In contrast, FDG-PET can offer relevant clinical information regarding treatment response early in the course of therapy. PET imaging can easily visualize these changes in tumor metabolic activity and indicate, sometimes within hours of the first treatment, whether or not a patient will respond to a particular therapy. Although treatment stratification based on metabolic response is an exciting potential application of PET, specific PET response assessment criteria still need to be developed and validated based on patient outcome

before changes in treatment regimens can be implemented.

Imaging tumor cell proliferation with PET is a new approach particularly attractive for cancer treatment monitoring as the target is a key feature of malignancy. Thymidine is utilized by proliferating cells for DNA replication and [F18]Fluoro-3'-deoxy-3'-L-fluorothymidine (FLT) has been developed as a suitable PET tracer. Changes in tumor blood flow and tumor cell proliferation are further encouraging approaches for predicting response. Ultimately, prediction of the therapeutic effectiveness by PET and PET/CT imaging could help to individualize treatment and to avoid ineffective chemotherapies, with their associated toxicities.

SPECT Imaging of Tc-99m Radiolabelled Polymers

Urs O. Hafeli and Katayoun Saatchi, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada

Microspheres and nanospheres made from biodegradable polymers are used for both controlled drug release and targeted drug delivery. During the development of such particles it is beneficial to be able to follow their fate *in vivo*, both qualitatively and quantitatively. An elegant method of tracking the particles over time from outside of an animal or patient consists of radiolabelling them with a gamma-emitting radioisotope and then observing their biodistribution with appropriate imaging methods such as single photon emission computed tomography (SPECT).

To allow for the imaging of biodegradable microspheres, a derivative of the biodegradable polymer poly(L-lactide) was prepared with a chelating group able to bind an imaging radioisotope. Specifically, we synthesized the biodegradable polymer poly(L-lactide) conjugated to a 2-bis(picolyamine) ligand. This chelating polymer was radiolabelled with the gamma-emitting radioisotope technetium-99m (Tc-99m), which is readily available and is the most used radioisotope in nuclear medicine. The radiolabelling, radiochemical stability, and *in vivo* biodistribution of our microspheres sized 1 and 10 μm in diameter will be presented. Potential imaging applications for the smaller particles include liver and spleen imaging due to their uptake by the reticuloendothelial system (RES) and for the larger particles lung perfusion

imaging or detection of deep vein thrombosis.

Using SPECT/CT imaging, it was possible to pinpoint the *in vivo* behaviour of our microspheres in a dynamic way and with a minimal number of animals. The use of Tc-99m radiolabelled biodegradable polymers and their imaging by SPECT/CT is thus a practical pre-clinical tool for pharmaceutical investigations, especially for the optimization of target organ uptake and tissue concentrations. For therapeutic regimens with highly

toxic drugs, such as cancer therapy, imaging could become an integral part of the treatment procedure. In this way, treatment efficiency could be quickly observed and maximized, while toxic side effects are minimized.

An excellent resource about preclinical imaging with SPECT is available online at www.SPECT-CT.com.

Session II b

Assessment of Bioequivalence for Drugs Requiring Pharmacodynamic Measures or Clinical Endpoints

(SPONSORED BY McDOUGALL SCIENTIFIC)

Bioequivalence Assessment of Topical Dermatological Dosage Forms: “Official” and Investigative Methods

Isadore (Izzy) Kanfer, Rhodes University, Grahamstown, South Africa

The assessment of the bioequivalence of topical products not intended for absorption into the systemic circulation has presented a formidable challenge over the years. In particular, dermatological dosage forms such as creams, ointments, lotions and gels, apart from those containing topical corticosteroids, cannot readily be assessed for bioequivalence using “conventional” methodology and the only recourse to-date has been to undertake tedious, time consuming and expensive clinical trials for such products.

Although the human skin blanching assay (HSBA), also known as the vasoconstriction assay (VCA) has been successfully used for dermatological products containing topical corticosteroids and the methodology has found formal acceptance by a number of regulatory agencies, e.g. the US FDA amongst others, no methodology for the bioequivalence assessment of other topical dermatological products such as those containing

non-steroidal anti-inflammatory drugs, anti-fungals, antibiotics and antivirals has yet found favour with regulatory agencies.

Application of the HSBA, Tape Stripping (TS) and Dermal Microdialysis (DMD) for the assessment of bioequivalence will be described and the theoretical basis and prognosis for each technique will be discussed.

Bioequivalence of Dosage Forms Administered Orally but not Intended for the Systemic Circulation

Keith Gallicano, Watson Laboratories, Inc., Corona, CA USA

Oral products that act locally within the gastrointestinal (GI) tract include binding agents and those agents intended to treat infections, inflammation, and diseases of the GI tract. Bioequivalence strategies for these products depend on performance factors of the dosage form (immediate- or modified-release (delayed or extended) tablets and capsules, suspensions), drug substance (solubility, permeability), and excipients (e.g., in suspensions, interactions). Determination of bioequivalence can involve *in vitro* (dissolution and

binding) studies, pharmacokinetic (PK), pharmacodynamic, and clinical endpoint studies. Alternatively, gamma scintigraphy has been proposed as a means to demonstrate bioequivalence of the active drug substance at the local site of action. Although locally acting GI products are not intended for systemic circulation, they may have adequate absorption of the active drug substance or its metabolite(s) such that these analytes can be measured in plasma in PK studies. However, the role of PK studies in bioequivalence of locally acting GI products is controversial because the clinical or therapeutic effect at the site of action for these products precedes entry of drug into the systemic circulation (i.e., measurable plasma concentrations). Although PK studies are more sensitive than comparative clinical endpoint studies to formulation differences, clinical endpoint studies that include a placebo arm are often recommended for bioequivalence of locally acting GI products when plasma concentrations do not reflect drug efficacy or are highly variable owing to low concentrations. The Office of Generic Drugs, U.S. Food and Drug Administration, has published individual bioequivalence recommendations for several locally acting GI drugs, including *in vitro* phosphate binding studies for sevelamer HCl tablet, *in vitro* dissolution or clinical endpoint studies for vancomycin HCl capsule, PK and clinical endpoint

studies for mebendazole chewable tablet, *in vitro* dissolution and clinical endpoint studies for mesalamine delayed-release tablet and extended-release capsule, and *in vitro* dissolution and PK studies for balsalazide disodium (mesalamine prodrug) capsule. This presentation will focus on the different methods to establish bioequivalence using the above examples considering dosage form and drug substance solubility, discuss the typical designs and data analysis techniques for clinical endpoint studies, and highlight the use of endoscopic imaging for evaluating product performance for locally acting GI products.

Inhaled Corticosteroids

Murray Ducharme, Cetero Research, Cary, NC

[No Abstract]

Clinical Endpoint Studies to Include Ophthalmics, Dermatologicals, and any Other Such Products

Grier Harris, Appian International, Charlotte, NC, USA

[No Abstract]

Session III a

Inter-Subject Variability in Drug Design and Pharmacotherapy

Rich Versus Sparse Sampling of Patient's Adherence to Pharmaceutical Product Candidates: What is Needed for Optimal Drug Development?

Bernard Vrijens, University of Liège, Belgium

Variable adherence to prescribed drug dosing regimens is prevalent among ambulatory patients, including those who participate in various types of controlled clinical trials. Variable adherence means variable exposure to prescribed or protocol-specified drugs. Variable exposure means dose- and time-dependent variability in drug actions, depending on the temporal pattern of drug exposure and the PK/PD of the drug in question. The extreme case occurs in patients who take no drug at all, but manage to camouflage that fact by manipulating the methods that still are routinely used to assess trial patients' exposure to protocol-specified drug dosing regimens. The primarily-used methods are counts of returned, untaken dosage forms and patient self-reporting. These have been repeatedly discredited as grossly overestimating adherence, based on direct comparisons with results of chemical marker methods and electronic medication-event monitoring (EM), both virtually free from post hoc manipulation by patients or caregivers. Chemical marker methods prove dose ingestion, quantifying aggregate drug intake over a period of a few days to a week, depending on the PK of the marker substance (e.g., low-doses of digoxin or phenobarbitone). EM time-stamps, stores, and communicates package entry times, which of course do not prove ingestion. Wide experience with EM, however, has shown it to be a robust indicator of dose ingestion, based on one's ability to use EM data to project the time course of drug concentrations in plasma, with direct chemical measurements confirming their accuracy in the vast majority of instances. Such confirmation is the gold-standard test of any method purported to compile drug dosing histories. EM is the only method that meets this stringent criterion.

But that is not the only issue in comparing

various methods for assessing ambulatory patients' exposure to prescribed drugs. The rate of data sampling is another, crucial issue. The rate of data sampling by EM is 4/h (96/day). In contrast, the widely-used method of auditing prescription refill intervals has a rate of data sampling determined by the interval between refills, typically are 1-3 months, i.e., a rate of data sampling of 4-12/year. Refill audits can reveal shortfalls in aggregate drug intake, and provide fuzzy estimates of when dosing may have stopped prior to an unfilled prescription. The same is true of other methods – interviews, questionnaires, occasional drug-level measurements – that occur in conjunction with scheduled clinic visits, i.e., a rate of data sampling of 1-12/year.

Not surprisingly, it has been only from EM data that we have learned the main errors that ambulatory patients make in their daily use of prescription or trial drugs: day-of-week/time-of-day patterns of dose omissions, times of occurrence of: single or sequential dose omissions (drug holidays), complete cessation of dosing (short persistence), pre-visit surges in adherence (white-coat adherence), mis-timed dosing. Such data provide valuable 'handles' for medication management and reveal to trial analysts what is probably the biggest single source of variance in drug response. The link between accurately-timed lapses in dosing and the subsequent time-course of drug concentrations in plasma and drug actions can give invaluable insight into key pharmacometric parameters. When such data are unavailable, one is left with unaccountably small drug responses and large variability in drug responses – and corresponding loss in statistical power. Reliable dosing history data and their pharmacometric covariates convert noise into signal, thus enriching the learning phase of drug development and avoiding type II errors in terminating product candidates.

Disease-Drug Interaction, a Source of Inter-subject Variability and Therapeutic Failure

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

Inter-subject variability is a major source of failure in both pharmacotherapy and clinical trials. Age, sex, race, disease and environmental variables are among the known factors that contribute to the inter-subject variability in response to drugs. However, the influence of diseases and conditions, other than those that the drug is intended for, have received very little attention. For example, inflammation is associated with many conditions such as various types of arthritis and cardiovascular diseases, cancer, inflammatory bowel diseases, infection, diabetes, aging and obesity. Consequently, mortality after myocardial infarction is associated with the inflammatory state of the patient. This may be, at least, in part, attributed to reduced response to pharmacotherapy as inflammation results in diminished potency of drugs. There are, however, emerging evidence suggesting that the altered response or pharmacokinetics may be correlated with the disease severity or with readily measurable inflammatory mediators. Correcting for the severity of inflammation as a covariate substantially improves the outcome of pharmacotherapy and clinical trials. Indeed, in clinical trials, smaller population sizes may be required to reach statistically sound conclusions if the covariate is considered. An understanding of the factors that contribute to inter-subject variability can improve pharmacotherapy and save cost and time of research and development.

A “Bottom-Up” Approach to Predict Inter-individual Variability in ADME

Amin Rostami-Hodjegan, Department of Human Metabolism, University of Sheffield, Sheffield, UK

Patients respond to drugs with a considerable variability. Understanding the sources of such variability and dealing with them is an inevitable

part of any drug development program as well as clinical management of drug therapy. *A priori* identification of influential characteristics leading to variations in response might improve the design of studies during drug development (with implications for reducing costs and the likelihood of “failure” due to inappropriate dosing strategies) and assist with better management of drug treatment for individual patients (i.e. personalised medicine).

An important component of variability relates to the absorption, distribution, metabolism and excretion (ADME) processes which govern the pharmacokinetics. Currently, the most common approach in identifying patient characteristics that influence ADME is the use of ‘population pharmacokinetics’ (POPPK). This is based primarily on pharmacostatistical considerations for the effects of parameters such as age, sex, body size, kidney function, genotypes of certain receptors or enzymes. The use of physiologically-based pharmacokinetics (PBPK) has created an alternative to traditional POPPK. The ‘bottom-up’ mechanistic modelling and simulation using PBPK is in line with all other ‘systems approaches’; it relies on first principles in building the system and is capable of identification and quantification of covariate effects. This follows since mechanistic PBPK takes account of biological variables known to be relevant to each step of process before the drug is studied *in vivo* [1, 2].

Examples of complex (non-linear) covariate effects are, particularly in paediatrics where body size cannot easily define the observed non-monotonic changes with age, will be demonstrated during the presentation. Overall, this presentation indicates that a framework for assessing inter-individual variability in pharmacokinetics using virtual human populations exists which integrates the general knowledge of physical chemistry, biology, anatomy, physiology and genetics to recognize the relevant covariates [1, 2].

[1] Rostami-Hodjegan A. and Tucker GT, *Nature Rev. Drug Dev.*, **6**: 140-8 (2007)

[2] Jamei M., Dickinson G.L., and Rostami-Hodjegan A., *Drug Metab Pharmacokin*, **24**: 53-75 (2009)

Session III b

Transdermal Drug Development

Recent Developments in Transdermal Drug Delivery

Michael Roberts, School of Pharmacy & Medical Sciences, University of South Australia and School of Medicine, University of Queensland, Australia

The skin is both our major barrier to our harsh external environment and a window to the health of our inner body. Drug delivery is and remains focussed on cosmetic, dermatological and systemic endpoints with a great emphasis now being placed on the use of nanotechnology and its safety after application to the skin. Our own work has emphasised both traditional principles in drug delivery, application of modern technologies such as nanopatches and the role of alterations in skin physiology. Solute size is a key determinant for penetration of solutes across the main skin barrier, the outermost stratum corneum layer. Perturbation of this layer by ultrasound, microdermabrasion, iontophoresis or microneedles can greatly enhance penetration. Our work has shown that skin blood flow causes highly bound solutes to be delivered to deeper layers of the dermis whereas transport of poorly bound solutes is mainly by diffusion in the dermis. Most recently, we have started to directly image events in occurring in the epidermis using multiphoton microscopy. This has allows us to examine transport in vivo and to look at influences such as flexing on nanoparticle penetration through human skin in vivo. In addition, we are now starting to examine how topical therapies affect the redox state of the skin.

The Design of a Skeletal Muscle Regenerator Molecule that is Applied Transdermally

Judy E Anderson, Faculty of Science and Frank J Burczynski, Faculty of Pharmacy, University of Manitoba, and G Wang, McColl-Lockwood Laboratory, Carolinas Medical Center

Nitric oxide (NO) mediates activation of skeletal

muscle satellite precursor cells to enter the cell cycle. This provides new precursor cells for skeletal muscle growth and muscle repair from injury or disease. Targeting a new drug that specifically delivers NO to muscle has the potential to promote normal function and treat neuromuscular disease, and would also help to avoid side effects of NO from other treatment modalities. In this research, we developed guaifenesin dinitrate (GDN) as a new NO donor for delivering nitric oxide to muscle, and tested in vitro for proof of principle before testing the effect on skeletal muscle cell activation via transdermal treatment. Normal adult mice were shaved over the dorsal thorax and GDN was applied in a cream base for 24 hours. Following euthanasia, muscles were dissected from the back region (quadratus lumborum), directly under the region of application, and from the hind limb (quadriceps) and homogenized for scintillation count assay of ³H-thymidine incorporation into DNA 24 hr later. Results showed there was a strong increase in muscle satellite cell activation and proliferation, demonstrated by a significant 38% rise in DNA synthesis in both muscles. Oral treatment of GDN confirmed the effect on myogenesis 24 hours after administration. This research showed systemic effect on muscle tissue, and extends the possibility that such treatment may be useful in promoting muscle repair in neuromuscular disease and after injury or in promoting muscle growth in atrophic conditions such as disuse and aging.

This work was supported by a Proof-of-Principle grant from the Canadian Institutes of Health Research.

(CIHR), a grant from the Manitoba Institute of Child Health and post-doctoral fellowships (to GW) from Manitoba Health Research Council and the CIHR.

Transdermal Aspects of Concurrent Use of Insect Repellent and Sunscreen Preparations: An Update

Xiaochen Gu, Faculty of Pharmacy, University of Manitoba, Winnipeg, MB, Canada

Increased public awareness for the protection against West Nile virus and skin cancer has led to extensive applications of topical insect repellent and sunscreen preparations. Concurrent use of both repellents and sunscreens has now become a common summer practice in Canada and the US. Combined repellent/sunscreen products are also commercially available to provide application convenience and dual protection. Designed as “Topical Use Only” preparations, however, active insect repellent and sunscreen ingredients should remain on the surface of skin with minimal systemic transdermal absorption. There are numerous active insect repellent and sunscreen agents used in the consumer-care products. In our laboratory, we have observed a synergistic percutaneous permeation between the repellent DEET and the sunscreen oxybenzone when

they were applied simultaneously, both in vitro and in vivo. These percutaneous characteristics are neither desirable nor productive for the topical skin preparations. Recently, we also carried out experiments to evaluate the transdermal characteristics of newer repellent picaridin and sunscreen oxybenzone. No mutual permeation enhancement was found between picaridin and oxybenzone when used concurrently. Percutaneous permeation of picaridin and oxybenzone was dependent on the preparation type and use concentration. In addition, overall permeation of picaridin was smaller than that of DEET, indicating an improved candidate for combined repellent/sunscreen formulations. Further studies are still ongoing to understand the percutaneous mechanisms and interactions between active insect repellent and sunscreen ingredients. Regular use of repellent and sunscreen preparations is one of the most effective and convenient methods to protect against vector-borne diseases and skin cancer.

Session IV a

Innovative Development of Drug Delivery

Development of a New Breath-Activated Powder Inhaler to Deliver Pure Drug and Higher Respiratory Fraction

Jesse Zhu, Particle Technology Research Centre, University of Western Ontario, London, ON, Canada

Pulmonary drug delivery is a much more effective and efficient method than taking drugs through the digestive system, and a much more convenient and safer method than injecting drugs intravenously. Human lung has a surface area of 80-100m² and delivering drug through the lung can short-cut the digestive system so that the delivery is very effective and only a small fraction of the normal drug dose is required. To realize pulmonary deliver, the drug powder must be smaller than 5 microns. Such superfine drug powders, however, cause a serious problem during packaging and administration, due to strong inter-particle forces. The inter-particle forces cause agglomeration and other flow problems, so that the delivery efficiency into the lung is limited to about 20% at most, with the existing inhalation devices.

Utilizing a patented rotating fluidized bed powder dispensing device, superfine drug particles in the range of 1-5 microns can be effectively suspended in gas stream and then metered into blisters that can be charged directly into dry powder inhalers. No large particle is required so that these superfine drugs can be used alone. The first key achievement of the new technology is the ability to accurately dispense a tiny quantity (in the order of 0.02 to 0.5 milligrams per dose) of the superfine drugs (< 5 microns). The second key achievement is the development of a more effective breath-activated inhaler that gives much higher delivery efficiency (> 50%) than those currently on the market. Test results with the new dispensing-inhaler will also be presented, including the initial animal study. A historical review on the past and existing designs of dry powder inhalers will also be given.

Overview of Gastric Retentive Technologies to Provide Once-a-Day Dosing for Drugs with a Narrow Absorption Window

Verne E. Cowles and Eddie S.Y. Hou, Depomed Inc., Menlo Park, CA, USA

Oral drug administration remains the predominant and preferred route for delivery of medications. However, due to incomplete absorption of many drugs in the lower gastrointestinal (GI) tract extended-release (ER) dosage forms must be maintained in the upper GI tract, preferably the stomach, while the medications are delivered to the region of the GI tract where they are best absorbed. Over the past two to three decades many approaches to gastric retentive ER drug delivery systems have been developed and tested. Among these are systems that depend on size (large single units), low (floating) and high (sinking) density, bio-adhesion (single and multi unit), and swelling. AcuForm[®] gastric retentive delivery technology is Depomed's unique, patented, polymer-based technology designed to optimize drug delivery. AcuForm allows for targeted, controlled delivery of pharmaceutical ingredients to the upper GI tract. Unlike immediate-release and some ER formulations that pass through the upper GI tract in approximately three hours following ingestion, AcuForm's unique swelling polymers allow the tablet to be retained in the stomach for a prolonged period of time following a meal. AcuForm tablets administered in the fed state have been shown to provide therapeutic advantages in two marketed products, with the duration of delivery characterized with respect to food and tablet size. Glumetza[®] (metformin hydrochloride extended-release tablets) is a diffusional-based swelling tablet demonstrating once-daily efficacy with good GI tolerability, while Proquin[®] XR ciprofloxacin hydrochloride extended-release tablets) is an erosional matrix that delivers the drug to the upper GI tract over 6 hours to provide once-daily efficacy with reduced incidences of nausea and diarrhea. The diffusional metformin

tablets consist of higher molecular weight hydrophilic polymers with polymeric swelling being faster than polymeric erosion. Consequently, the drug is released from the tablet by diffusion linearly with the square root of time and the tablet may enlarge up to three times its original size promoting gastric retention. Although the drug is released in approximately 8 h, the polymer erodes nearly linearly over 12 – 15 h so that ghost tablets are generally not eliminated in the feces. For the erosional ciprofloxacin formulation consisting of lower molecular weight hydrophilic polymers, the tablet enlarges initially from polymeric swelling. It then predominantly maintains its size with a slow linear decrease by polymeric erosion and a sharp decrease in size near the end of drug delivery. The polymeric erosion results in nearly linear delivery of the ciprofloxacin over 6 h, with the rate of delivery being under predicted by dissolution testing and more accurately predicted by disintegration.

Sono-Crystallization, Particle Engineering, and Improved Performance of Inhalable Pharmaceutical Particles

Graham Ruecroft, Prosonix Ltd., Oxford Science Park, Oxford, UK

Controlling and actively manipulating crystal nucleation and subsequent growth behaviour is fundamental to the improvement of pharmaceutical crystallization and crystal engineering processes for active pharmaceutical ingredients (APIs). This control is necessary when the final product performance is reliant upon having well-defined and engineered mesoscopic particles, such as in inhaled medicines designed for asthma, COPD and infection for example. New advanced particle engineering technologies utilising power ultrasound (so called sono-crystallization) span all aspects of the pharmaceutical pipeline including discovery, process optimisation, manufacture, formulation and drug

delivery, and in doing so can help realise better medicines. In respiratory drug delivery, effective deep lung deposition is achieved with particles of 1 to 5 microns in size. To achieve optimal drug delivery to the lung it is important to ensure that the drug is formulated into particles with the appropriate aerodynamic size, shape, density, surface properties and stability. Sono-crystallization has been applied to many compounds for inhalation including steroidal asthma drugs (such as budesonide and fluticasone propionate), bronchodilators (such as salmeterol xinafoate and formoterol fumarate), antibiotics (such as tobramycin), and many new chemical entities under development. For single drug substances the aerosolization efficiency, using the NGI (Next Generation Impactor), of various sono-engineered steroidal and bronchodilator drug substances have been assessed in binary (with micronized lactose) dry powder inhaler (DPI) formulations and in all cases were 50-120% more efficient in terms of Fine Particle Dose and certainly superior in terms of emitted dose. Furthermore, there is a real drive to produce combination particles whereby two or more APIs, in an exact ratio, can be converted to single particles containing the very same drug substances as separate crystalline entities. In combination therapies for asthma and COPD the APIs often have synergistic action at molecular and cellular level and need to be delivered in an exact ratio to the site of action in the lung. In Seretide (FP and SX) for example the two APIs can be symbiotically sono-crystallized into single particles. In all cases, as well as improved particle stability, the Fine Particle Dose was consistently higher (> 30%) than when using individual micronized powders. With sono-crystallized and well-engineered single and combination particles asthma and COPD treatment may undergo some significant changes with respect to both the engineered APIs and actual delivery devices.

Session IV b

PAT/QbD – Are We Ready?

Pharmaceutical Development - Building Robust Product

Krishnan Tirunellai, Therapeutic Products Directorate, Health Canada, Ottawa, ON, Canada

ICH guideline Q8 (R1) highlights the important concepts of pharmaceutical development. It also provides an opportunity to establish a higher degree of confidence in product quality, through the Quality by Design (QbD) approach. In this presentation the speaker will discuss the role of Process Analytical Technology (PAT) as a tool to build a high quality product through a robust process. An update on ICH activities related to the implementation of Q8, 9, and 10 will also be provided.

Industrial Perspectives of PAT

Ricardo Vargas, Formulations and Processes, Purdue Pharma, Pickering, ON, Canada

Process Analytical Technology (PAT) and Quality by Design (QbD) are the latest trends in the pharmaceutical industry. In the last ten years, pharmaceutical companies have placed a lot effort to improve how their products are being developed and manufactured as means to have processes that are in control. As a result, several different technologies have been used to gain more process knowledge to understand what happens during the manufacturing and testing of a product. PAT has become an essential component of Quality by Design, which encompasses the process knowledge and process understanding of the product a company develops and manufactures. This knowledge allows one to establish the key quality attributes and the key process parameters that are critical to the process to maintain the SISPQ (Safety, Integrity, Strength, Purity and Quality) of the product. Moreover, companies are able to concentrate on those parameters and establish the design space which yields a product with high process capability (Cpk) values > 1.33; by doing that, companies ensure their

processes are more efficient and more predictable with an end product of the highest possible quality. Furthermore, that translates into considerable savings during the product life cycle time.

Process Analytical Technology consists of all the different tools and technologies that provide the information required to gain the knowledge and understanding about the process and the product. This information would help one to execute and maintain a process under control by adjusting the process variables according to the characteristics of the input variables. At the end, one can be certain prior to the testing of the finished product that it would meet all the required release specifications.

It is important for a company that is looking to implement PAT-QbD to have a plan but more importantly to spend enough time to evaluate all the tools and technologies that are available to determine the one(s) that are more suitable to the company's products and processes to obtain the best return on investment.

Session V

Current Regulatory Issues on the Determination of Bioequivalence

Bioequivalence of Complex Drug Products: Regulatory Science and Its Implications for Patient Care

Bruce D. Clark, Apotex Inc., Toronto, ON, Canada

Bioequivalence is the established methodology for proving therapeutic equivalence of a new or modified formulation of a drug product. It is the globally accepted criteria for ensuring that a generic product is equally safe and efficacious as the brand product. Generic medicines provide substantive saving for insurers as well as the provinces resulting in savings that can, in part, ensure that funds continue to be available to pay for new drugs.

Current bioequivalence requirements work well for both uncomplicated and complicated drugs including those with narrow therapeutic index, nonlinear pharmacokinetics and products with modified release formulations. However, for drugs or drug products with high inherent variability, the conventional criteria for bioequivalence have been shown to be problematic. In situations where high variability is caused by an inadequate formulation of the brand product, generic companies face the dilemma of having to produce an equally inadequate formulation to match the brand.

Locally acting drugs where typical bioequivalence studies with pharmacokinetic measurement are not adequate for ensuring therapeutic equivalence of a generic product are also problematic. Examples of these drugs include topical products, ophthalmic suspensions, orally inhaled products for asthma and COPD, and drugs with local action in the gastrointestinal tract. They often require comparative safety and efficacy studies or a bioequivalence study with a pharmacodynamic endpoint. Many of these studies are much more expensive to conduct and take a much longer time to complete than the traditional PK bioequivalence studies. As a result, it is critical that clear regulatory guidance for the design of these studies and the equivalence criteria be available promptly so that the appropriate studies will be conducted and products

can get to market quickly.

Biopharmaceuticals are now an increasingly important part of the healthcare system. Because of their complex structure, the demonstration of bioequivalence for these products requires complicated physico-chemical characterization in addition to PK bioequivalence studies and clinical safety and efficacy studies. Immunological and animal toxicity studies may also be required. These are a tremendous investment for a subsequent entry or biosimilar product. With the complexity involved, it is understandable that the establishment of regulatory guidance for these products is difficult. It is important that regulatory authorities work with the scientific community and drug industry to come up with requirements that not only ensure the biosimilar products to be equally safe and effective as the original brand products but are also financially feasible to meet even for small markets like Canada.

Implementation of the BCS Guidance: The US FDA Experience

Mehul Mehta, Office of Clinical Pharmacology, OTS, CDER, FDA, Silver Spring, MD, USA

The central principle behind the Biopharmaceutical Classification System (BCS) is that if a drug substance is highly soluble (HS) and highly permeable (HP), and the immediate release formulation of this drug is rapidly dissolving (RD) over the physiological pH range, then different (rapidly dissolving) formulations of such a drug will have minimal impact on the rate and extent of its systemic bioavailability. The US FDA issued a final guidance on this topic nearly 9 years ago, in August 2000. The key feature of the guidance was that for a BCS Class 1 product, i.e., for a HS, HP, RD product, the guidance allowed waiver of in-vivo bioequivalence studies based entirely on adequate in-vitro dissolution results. Soon after the issuance of this guidance, the Center for Drug Evaluation & Research (CDER) formed the BCS Committee for

consistent application of this guidance on the new drug side as well as generic drug side, and also to periodically assess progress in this area and evaluate the need to update the guidance. Since its formation, the committee has met on a regular basis and evaluated a large number of applications, on both the new as well as the generic drug sides that have sought BCS based biowaivers. This presentation will cover the process and functioning of the committee, how applications are evaluated, number of applications evaluated and granted BCS Class 1 status. A few examples will be presented to highlight complex issues faced in evaluation and their resolution. The presentation will close with current debate in literature on this topic and some thoughts on possible areas of the guidance that could be considered for updating.

Evaluating Bioequivalence for Difficult Drug Products

Barbara Davit, Office of Generic Drugs, CDER/FDA, Rockville, MD, USA

In the US, an applicant seeking marketing approval of a new generic drug must submit to the FDA evidence that its product is bioequivalent to – has the same rate and extent of drug absorption at the site of action as – the corresponding reference product. This means that, for systemically active oral dosage forms, bioequivalence can usually be demonstrated in an in vivo pharmacokinetic study in which rate and extent of drug absorption are evaluated by the parameters C_{max} and AUC. The generic and reference product are deemed bioequivalent if the 90% confidence intervals of the geometric mean C_{max} and AUC ratios fall within the limits of 80-125%. However, there are some classes of drug products for which different approaches are more

suitable for establishing bioequivalence. These products include formulations of locally acting drugs, drugs with highly variable bioequivalence parameters, and combination immediate-release/modified release formulations. The objectives of this presentation will be to present approaches under consideration by the US-FDA for establishing bioequivalence of these difficult drug products.

Updated Guidance for the Conduct and Analysis of Bioequivalence Studies

Eric Ormsby, Therapeutic Products Directorate, Health Canada, Ottawa, ON, Canada

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 are being developed by Health Canada which will combine the 10 active guidances on our website. It will combine bioequivalence guidances Part A and Part B into one conduct of bioequivalence studies. This guidance allows for different collection of data methods based on sequential and adaptive designs as well as the use of add-ons. The guidance also suggests what an extreme value is and various methods to account for or remove these extreme values. The second guidance will define various classes of drugs and their respective bioequivalence standards. This guidance defines critical dose drugs, non-linear drugs and highly variable drugs and what studies and standards need to be carried out in order to determine bioequivalence. This talk will outline the proposed changes to Health Canada's approach to bioequivalence determination.

Speaker Biographies

Christine Allen

Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada

Christine Allen is an Associate Professor in the Faculty of Pharmacy at the University of Toronto. She is cross-appointed in the Departments of Chemistry, Chemical Engineering and Applied Chemistry and the Institute of Biomaterials and Biomedical Engineering. Her research is focused on the rational design and development of new materials and technologies for the delivery of drugs and contrast agents (Lab Website: <http://phm.utoronto.ca/~allen/>). Allen completed her doctoral research in the Department of Chemistry at McGill University and post-doctoral research in the Department of Advanced Therapeutics at the B.C. Cancer Agency. She joined University of Toronto in 2002, from Celator Pharmaceuticals Inc. (Vancouver, B.C.) where she had worked as a scientist and Assistant Director of materials research. She has over 50 publications, numerous patent applications, and six book chapters on both lipid and polymer-based delivery systems. She has served on several peer review panels for granting agencies including CIHR, NCIC and NIH. In 2004, she was awarded a CIHR-Rx&D Career Award (2004-2009) for her research on the design and development of technologies for cancer treatment. In 2006, she was awarded the Association of Faculties of Pharmacy of Canada/AstraZeneca New Investigator Research Award and the Canadian Society Pharmaceutical Science/GlaxoSmithKline Early Career Award. She was the host and Chair of the 6th International Nanomedicine and Drug Delivery Symposium held in Toronto 2008.

Gordon L. Amidon

College of Pharmacy, The University of Michigan, Ann Arbor, MI USA

Dr. Gordon L. Amidon received his B.S. degree from the State University of New York, Buffalo (1967), an M.A. degree in Mathematics (1970) and PhD in Pharmaceutical Chemistry (1971) from The University of Michigan. From 1971 to 1981 Dr. Amidon was a member of the faculty at the University of Wisconsin. Dr. Amidon was appointed Professor of Pharmaceutics at The University of Michigan in 1983 and was named the Charles R. Walgreen, Jr., Professor of Pharmacy in 1994. Dr. Amidon is internationally known for his

research in the field of drug absorption, transport phenomena, solubility and dissolution, and prodrugs. He has published extensively in journals, with over 280 published papers and 370 abstracts, 18 US patents, contributed chapters to over 30 books and monographs and is co-editor of eight books. Professor Amidon has mentored over 80 doctoral and postdoctoral students with more than 20 selecting academic careers. He has received numerous awards including; best paper awards in the *Journal of Pharmaceutical Sciences* (1975, 1981, 1984) and *Pharmaceutical Research* (2004); the Scheele Award of the Swedish Academy of Pharmaceutical Sciences for outstanding contributions to the field of oral drug delivery and biopharmaceutics (1996). He received an honorary Doctor of Pharmacy degree from the University of Uppsala, Sweden (2001); the Founders Award of the Controlled Release Society (2003); the Volwiler Award of the American Association of Colleges of Pharmacy (2004); the AAPS Distinguished Pharmaceutical Scientist Award (2005); the FIP Distinguished Pharmaceutical Scientist Award (2006), and the Gerhard Levy Distinguished Lectureship (2006). He has organized and participated in many international symposia and workshops. Dr. Amidon developed a Biopharmaceutics Classification System (BCS), with the FDA, impacting bioequivalence standards worldwide. He is a Fellow of the AAPS, APhA/APS, and the AAAS. He is a member of the Controlled Release Society, serving as president in 1994, AACP, ACS and AAPS, serving as president in 1998. Professor Amidon is the editor of the American Chemical Society Journal, *Molecular Pharmaceutics*.

Judy Anderson

Biological Sciences, Faculty of Science, University of Manitoba, Winnipeg, MB, Canada

Dr. Anderson (BSc Zoology, University of British Columbia; BSc Medicine, University of Manitoba; PhD, Human Anatomy, University of Manitoba) is a professor at the Faculty of Science at the University of Manitoba. She joined faculty in 1988 in the department of Anatomy, and served as Associate Dean (Academic Affairs) in Medicine (2003-2006), Acting Head of Pathology (2005) and briefly as Acting Dean of Medicine (July-Sept 2004). She has been the Head of Biological Sciences in the Faculty of Science since October 2007. She also serves on

university Senate and Board of Governors.

Her research interests include the cell and molecular biology of muscular dystrophy pathophysiology, myogenic regeneration, and muscle atrophy and growth, as well as anatomical basis of muscle rehabilitation, and collaborative research on development of interprofessional education. The basic science aspect of her work has included discovery of the role of nitric oxide in mediating satellite/stem cell activation in skeletal muscle, which led to collaboration to develop a new formulation to prevent or treat muscle atrophy and promote repair. Trainees in BSc (Honours), MSc, and PhD programs have contributed to developing a systems approach to muscle structure and function and into the translation of research in drug development, education, and rehabilitation. Funding for these projects has ranged from CIHR and the Muscular Dystrophy Association to the Canadian Space Agency and Health Canada.

For her teaching and mentoring of graduate students, she received the University of Manitoba Graduate Students Association Graduate Teaching Award (2001) and the Health Sciences Students Association Distinction in Mentorship award (2001).

Stephane Angers

Faculty of Pharmacy & Department of Biochemistry, University of Toronto, Toronto, ON, Canada

Dr. Angers (1997- B.Sc Biochemistry, McGill University, Canada; 2002- Ph.D, Biochemistry, Université de Montréal, Canada) is a Professor at the Faculty of Pharmacy and in the Department of Biochemistry at the University of Toronto. He joined the University of Toronto in October 2006 after a post-doctoral fellowship in the Howard Hughes Medical Institute laboratory of Dr. Randall T. Moon at the University of Washington in Seattle.

His research interests include the mechanisms of signal transduction by cell surface receptors. Current projects in his laboratory aims at better understanding intracellular signalling initiated by the Wnt and Hedgehog family of secreted proteins as well as by the activation of G protein coupled receptors during development and human diseases. Using novel proteomic approaches his lab identifies and functionally characterizes novel protein-protein interactions underlying these signalling pathways. During his young career Dr. Angers has published several high impact refereed articles in journals such as Nature, Science and Nature Cell Biology. He

holds the Canada Research Chair in Functional Architecture of Signal transduction.

Norbert Avril

Department of Nuclear Medicine, Barts and The London School of Medicine, Queen Mary College, University of London, London ,UK

Norbert Avril is Director of the PET Centre and Lead Consultant in Nuclear Medicine at Barts and The London School of Medicine. After his training in Nuclear Medicine at the Technische Universität in Munich, Germany, he was a research fellow at Memorial Sloan-Kettering Cancer Center in New York exploring the development and validation of reporter gene - reporter substrate combinations for radionuclide imaging of transgene expression. From 2002 to 2005 Dr. Avril was Chief of the Division of Nuclear Medicine at the University of Pittsburgh, USA. The focus of his research is the use of Positron Emission Tomography (PET) in oncology. Several recent research projects addressed the use of PET/CT for radiation treatment planning. His particular interest is in developing new molecular imaging strategies using PET and PET/CT to optimise cancer treatments. The current research activities focus on the evaluation of molecular PET imaging as a new surrogate endpoint for assessment of early treatment effects and prediction of therapeutic outcome. This includes both established and new targeted therapies such as gene therapy. He is member of the Society of Nuclear Medicine (SNM) and the European Association of Nuclear Medicine (EANM) and Editorial Board member for The Journal of Nuclear Medicine, Molecular Imaging and Biology (Associate Editor) and BMC Medical Physics (Editor).

Reina Bendayan

Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada

Reina Bendayan is a Professor and Associate Dean Graduate Studies, Graduate Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto. After obtaining a Bachelors of Sciences in Pharmacy and a Hospital Pharmacy Residency Program at the University of Montreal, Reina Bendayan completed a Doctor of Pharmacy at the University of Florida and a three year Medical Research Council Post-Doctoral Fellowship Program in Clinical Pharmacology and Membrane Cell Biology at the University of

Toronto. Dr. Bendayan's research program at the University of Toronto is primarily focused on Membrane Transport and Therapeutics with an emphasis in the field of HIV/AIDS Antiviral Drug Transport. Her research is primarily funded by the Canadian Institutes of Health Research, Canadian Foundation for AIDS Research and the Ontario HIV Treatment Network, Ministry of Health of Ontario. She is a member of several scientific associations, in particular AAAS, ASPET, AAPS, ASCPT, IAS, CAHR, CSPS. In 2006-07, she served as the Chair of the AAPS Drug Transport Focus Group. She has participated to the organization of several Workshops and Symposia for International and National Pharmaceutical Sciences Conferences as well as Gordon Conferences on "Barriers of the CNS". From January 1 to July 1, 2007, Dr. Bendayan served as Acting Dean of the Leslie Dan Faculty of Pharmacy.

Ron Boch

Pharmaceutical Consulting, North Vancouver, BC, Canada

Dr. Ron Boch is a pharmaceutical consultant with over ten years of industrial drug development experience, providing services in candidate selection, formulation, drug delivery and process development for manufacturing. Dr. Boch is a named inventor of more than sixty US and international patents and patent application filings including eight patent families. While working at QLT Inc., Dr. Boch contributed to the development of Visudyne that was approved for treatment of wet age related macular degeneration, a leading cause of blindness in people over the age of 55. As the Associate Director, Formulation at QLT, he was responsible for establishing and leading a multi-disciplinary team in the development of a wide range of products. In addition to his contributions in industry, Dr. Boch is an Honorary Research Associate with the University of British Columbia. As an NSERC Industrial Research Fellow, Dr. Boch worked in the Department of Chemistry at UBC with Dr. David Dolphin on the determination of structure-activity relationships of new drug candidates. Dr. Boch obtained his Ph.D. degree in Chemistry with Dr. J.C. Scaiano and a B.Sc. in Biochemistry from the University of Ottawa.

Frank Burczynski

Faculty of Pharmacy; and Department of Pharmacology & Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada

Dr. Burczynski (B.Sc. Pharm, M.Sc (Pharmaceutics), Ph.D. (Pharmacology), University of Manitoba) is a professor at the Faculty of Pharmacy, University of Manitoba. He joined the faculty at the University of Manitoba in 1992 and served as the associate dean research from 1999 to 2004. His main research interests include effect of hepatic pathophysiological changes on the uptake and intracellular disposition of drugs, and development of novel dosage forms. Dr. Burczynski's research work has been and currently is funded by the Canadian Institute of Health Research as well as the pharmaceutical industry. He has published over 120 refereed articles and has trained many undergraduate research students and MSc and PhD level graduate students. Dr. Burczynski teaches pharmacokinetics at the undergraduate and graduate level and is involved in pharmacy curriculum development.

Helen M. Burt

Faculty of Pharmaceutical Sciences, UBC, and Drug Delivery, Centre for Drug Research and Development, Vancouver, BC, Canada

Dr Burt is the Angiotech Professor of Drug Delivery and the Associate Dean, Research and Graduate Studies in the Faculty of Pharmaceutical Sciences at UBC. She obtained her Ph.D in Pharmaceutics in 1980 from UBC. Her research efforts are focused on the development of polymer-based drug delivery systems for controlled and localized drug delivery and in the synthesis and evaluation of new biodegradable polymers as suitable biomaterials or carriers for drugs. Her work is currently supported primarily by grants from the Canadian Institutes of Health Research (CIHR) and Natural Sciences and Engineering Research Council (NSERC). She is a co-founder and Division Head of the Division of Drug Delivery in the BC Centre for Drug Research and Development, a CFI-funded centre located at UBC. She has published over 120 peer-reviewed papers and numerous patents. She has been the recipient of several teaching prizes, including the Killam Teaching Prize in 1990. From 1997-2001, she chaired the faculty committee that designed and developed an innovative outcomes-based undergraduate curriculum for pharmaceutical

sciences. She received the YWCA Woman of Distinction Award for Science, Research and Technology in 2000, the NSERC Synergy Award for Innovation in 2006, the Association of Faculties of Pharmacy of Canada-Pfizer Canada Research Career Award in 2006 and was made a Fellow of the Canadian Academy of Health Sciences in 2005. Dr Burt has supervised over 35 graduate students/postdoctoral fellows/research associates and many undergraduate research assistants. She was the Health Research Coordinator in the Vice President Research Office at UBC from 2000-03 and worked with a team of individuals to create and implement the programs offered by the Health Research Resource Office (HeRRO) at UBC. She is a member of the Board of Directors of the Provincial Health Services Authority.

Jason Chittenden

Pharsight Corporation, Cary, NC, USA

Jason Chittenden joined Pharsight in September 2005 as Product Marketing Manager, spent time as Director, Training and Pre-Sales, and is currently Director, Product Quality. At Pharsight, he has been heavily involved in the development of the IVIVC Toolkit for WinNonlin and the next generation WinNonlin and NLME products. Prior to Pharsight, Jason was a Senior Scientist at Simulations Plus where he was the Product Manager for GastroPlus®. At Simulations Plus, Jason consulted on topics such as: absorption, PK/PD, and physiologically based pharmacokinetic (PBPK) modeling; In-Vitro In-Vivo Correlation (IVIVC); and bioequivalence studies. Jason has worked in simulation and modeling since 1997 and has experience in the chemical, petroleum, and pharmaceutical industries. He obtained his Master of Science in Chemical and Biochemical engineering from the University of California, Irvine, in 2000, where he focused on neural network and optimization algorithms and theory. He is currently a Ph.D. student (part-time) in the Biomathematics program at the North Carolina State University.

Kwok Chow

Patheon Inc., Mississauga, ON, Canada

Dr. Kwok Chow is the Senior Director of Global PDS Technology and Alliances at Patheon Inc. with the responsibility of developing/introducing new technologies, establishing strategic technical alliances and providing scientific input to support

the pharmaceutical development of challenging molecules. Previously, he was the Director of Formulation Development managing the services for the development of a wide range of formulations including those containing low solubility/bioavailability drug substances and/or requiring modified release characteristics.

Dr. Chow began his career at Glaxo where he was leading the development and design of a variety of conventional and novel dosage forms including tablets, capsules, liquids, suspensions, nasal sprays, powder for reconstitution and fast dissolving formulations. He played a key role in the introduction of a number of new chemical entities and line extension products in North America, Europe, Japan and other Asian Pacific countries. He also conducted and supervised solid-state pharmaceuticals as well as powder technology research.

Dr. Chow received his B.S. in Pharmacy from the University of Minnesota and his Ph.D. in Industrial Pharmacy with Professor David Grant from the University of Toronto on crystal modification and crystal engineering of drugs. Dr. Chow taught both undergraduate and graduate courses in formulation development at the University of Toronto. He also supervised industrial graduate students. He is the author of a number of patents, abstracts and research/review articles.

Bruce D. Clark

Apotex Inc., Toronto, ON, Canada

Dr Clark is Vice President of Regulatory and Medical Affairs for Apotex Inc. Based in Toronto, Apotex is Canada's largest generic pharmaceutical manufacturer. Dr Clark is responsible for global regulatory and medical affairs and an executive member of the Apotex management team. He is a member of both the Canadian and European Generic Pharmaceutical Association Subcommittees on subsequent entry biologics (SEB) or biosimilars and has participated in several international conferences and regulatory forums representing the industry views on development of guidelines for these products. Prior to joining Apotex, Dr Clark served as a consultant to the pharmaceutical and biopharma industry and held the positions of Vice President of Scientific Affairs at Sanofi synthelabo and Director of regulatory affairs for Glaxo Wellcome.

Verne E. Cowles

Depomed, Inc., Menlo Park, CA, USA

Dr. Verne Cowles joined Depomed, Inc in 1999 and is the Sr. Director of Preclinical Studies and Gastrointestinal Physiology. His current research is focused on developing gastric retentive extended-release dosage forms which reduce the dosing frequency of drugs that are absorbed in the upper gastrointestinal tract and may also reduced the side effects associated with these drugs. Prior to joining Depomed he was a Sr. Research Scientist at Abbott Laboratories in the Department of Integrative Pharmacology. He graduated from the University of Wisconsin with a B.S. in biology and chemistry, and received his Ph.D. in physiology from the Medical College of Wisconsin. Following graduation he was appointed to the faculty at the Medical College of Wisconsin in the Departments of Surgery and Physiology. He has been awarded research grants from the National Institutes of Health and the Department of Veterans Affairs. He has over 50 peer-review publications in the field of gastrointestinal physiology, and has written several book chapters on the subject. He is a member of the American Gastroenterological Association (AGA), American Neurogastroenterology and Motility Society, The International Group on Neurogastroenterology and Motility, Functional Brain-Gut Research Group, American Association of Pharmaceutical Scientists (AAPS) and the Control Release Society (CRS).

Barbara M. Davit

Office of Generic Drugs, CDER/FDA, Rockville, MD, USA

Barbara Davit, Ph.D., J.D., is the Acting Director of the new Division of Bioequivalence II in the Office of Generic Drugs, CDER/FDA. Her professional areas of expertise are pharmacokinetics and drug metabolism. Dr. Davit holds a B.S. in Chemistry from Georgian Court College, a Ph.D. in Nutrition Science from the University of California, Davis, and a J.D. from George Mason University School of Law. Dr. Davit trained in pharmacokinetics as a postdoctoral fellow at the California Primate Research Center. Following several years in the CRO industry, Dr. Davit joined CDER/FDA as a Reviewer in 1991. Within the Division of Bioequivalence, she served as Team Leader from 1998-2002 and as Deputy Director from 2002-2008. Dr. Davit contributes to CDER biopharmaceutic and

generic drug guidance development, provides technical assistance to FDA counsel when needed, and has co-authored several book chapters and original research articles on bioequivalence testing of generic drug products.

Murray P. Ducharme

Cetero Research, Cary, NC, USA

Murray P. Ducharme, Pharm D, FCCP, FCP is Chief Science Officer at Cetero Research, a North American contract research organization, where he is globally responsible for the regulatory and scientific affairs business of the company. He directs the work of PK scientists, statisticians, medical writers, and clinical pharmacologists dedicated at providing to both generic and innovator pharmaceutical companies all types of pharmacokinetic and statistical analyses necessary to do during the drug development process. These include bioequivalence, drug-drug interactions, thorough QTc, special populations, proof of concepts, and any types of modeling and simulation studies including population PK/PD analyses. He also serves as a Professeur Associé at the Faculté de Pharmacie, University of Montreal, where he directs the research work of doctoral and post-doctoral students in clinical pharmacology. Dr. Ducharme has authored or co-authored more than 150 articles, abstracts, book chapters and manuals. He has also presented more than 200 posters and seminars at conferences, symposiums, meetings and workshops.

Leanne Embree

LinkCore Pharma Corporation, Vancouver, BC

Dr. Embree has senior management expertise in the development of human drugs and devices. She earned her Ph.D. (1989) in pharmaceutical chemistry from the University of British Columbia, Vancouver, Canada. Following post-doctoral studies with the MRC Regulatory Peptide Group in the Faculty of Medicine at the University of British Columbia, Dr. Embree joined Medical Oncology to initiate the Investigational Drug Program at the British Columbia Cancer Agency in 1990. As part of the Investigational Drug Program, she implemented quality systems to enable drug development from bench-top through to Phase II clinical testing as well as pharmacokinetic assessment for early drug testing. In addition, her research activities include clinical therapeutic drug monitoring for busulfan which is used as preparative chemotherapy prior to

bone marrow transplantation and development of analytical methods for quantitation of platinum anti-neoplastic agents in blood. In 1997, Dr. Embree took on corporate responsibilities for R&D at Angiotech Pharmaceuticals, Inc. and expanded the departmental capabilities to support moving prototypes from early research to clinical testing. Dr. Embree provided strategic insight, leadership and direction to the R&D Department and, most recently, the Analytical Chemistry Department within R&D as the company grew from less than 30 in 1997 to around 1600 in 2007. Dr. Embree joined LinkCore Pharma Limited in 2007 where she currently has corporate responsibilities for R&D. In addition, Dr. Embree does consulting for early-to-late stage product development to drug and drug-device companies. She is a member of CSPS, AAPS, RAPS, DIA, AOAC and ACS and actively serves the profession as Past-President of CSPS. Dr. Embree has over 50 publications and presentations of her research.

Laszlo Endrenyi

University of Toronto: Toronto, ON, Canada

Dr. Endrenyi is Professor Emeritus of pharmacology and biostatistics in the University of Toronto. He has served the university in various positions including on its Governing Council and as Associate Dean of Graduate Studies. Externally, he has served on grant review committees and editorial boards of research journals including the Amer. J. Physiol, J. Pharmacokin. Pharmacodyn., J. Pharm. Pharm. Sci., and J. Pharm. Sci. He edited a book on Kinetic Data Analysis, and published over 150 research papers. Several of these provided principles for the design and analysis of enzyme and pharmacokinetic investigations. More recently, he extensively developed principles and applications for the evaluation of bioavailability and bioequivalence. He has consulted with the Food and Drug Administration and the Health Protection Branch and served on their advisory committees. He has consulted also with industry in the areas of pharmacokinetics, biostatistics, the design and evaluation of experiments, clinical trials, and the analysis of bioavailability and bioequivalence studies.

Raymond Evers

Merck & Co, Rahway, NJ, USA

Dr Raymond Evers is currently employed as a Director In Vitro Technologies in the Department of Drug Metabolism and Pharmacokinetics (DMPK) at Merck & Co. His main responsibilities are to study the propensity of drug candidates to cause pharmacokinetic drug-drug interactions due to enzyme inhibition or induction, or modulation of drug transporter activities.

Raymond Evers studied Molecular Biology at the University of Amsterdam where he also defended his Ph.D thesis based on work performed at the Max-Planck Institute for Biology in Tübingen, Germany. After completion of post-doctoral studies at the German Cancer Research Center, Heidelberg, and The Netherlands Cancer Institute, Amsterdam, in the areas of transcriptional regulation and multi-drug resistance, respectively, he was employed as a group leader at the Georg-Speyer-Haus in Frankfurt, Germany. In Frankfurt, he was leading a group focusing on studying the signals determining plasma membrane routing of multidrug resistance proteins.

Brian C. Foster

Therapeutic Products Directorate, Health Canada, Ottawa, ON, Canada

Dr. Foster is a Senior Science Advisor in the Office of Science, Therapeutic Products Directorate, Health Canada. He received his Ph.D. in Medicinal Chemistry at the University of Alberta through research on alternative models for drug interactions and metabolism. Since joining Health Canada, his research has been in the areas of toxicology and drug disposition. His current research interest is in the area of drug disposition, pharmacogenetics, and how natural health products affect the safety and efficacy of conventional therapeutic products. Dr. Foster is an Adjunct Professor, Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa where he has graduate students in a joint Health Canada - University of Ottawa Centre for Research in Biopharmaceuticals and Biotechnology laboratory.

Keith Gallicano

Biopharmaceutics Operations, Watson Laboratories, Inc., Corona, CA USA

Dr. Gallicano is Director, Biopharmaceutics Operations, Watson Laboratories, Corona, CA. He received his Ph.D. in chemistry from the University of British Columbia in 1980. Shortly thereafter he completed an industrial research fellowship investigating bioanalytical methods for the isolation, identification and quantitation of drugs in race horses. In 1986 he joined the Royal Canadian Mounted Police and trained as a Forensic Chemist, specializing in the analysis and comparison of materials, such as petroleum, paint, glass, building products, headlamps and explosives, from scenes of crime. From 1988 to 1997 Dr. Gallicano was a Research Scientist in the former Bureau of Drug Research, Health Protection Branch (HPB), Ottawa, where he pursued his interests in development and validation of bioanalytical assays and in clinical pharmacokinetic studies, particularly those involving drug interactions of drugs used in HIV therapies. In 1997 he left HPB as a senior Research Scientist and Head of the Biopharmaceutics and Pharmacokinetics Section to join the Clinical Investigation Unit, Division of Infectious Diseases, Ottawa General Hospital as a clinical research scientist and the University of Ottawa as an Assistant Professor of Medicine. He returned to Vancouver in 2000 as Director, Pharmacokinetics, Axelson Biopharma Research and then moved to California in 2003 to take on his current position.

Dr. Gallicano has co-authored 72 publications, including research papers, reviews, and book chapters. He was a member of the Editorial Board of the *Journal of Chromatography* and the *British Journal of Clinical Pharmacology*. Dr. Gallicano has given numerous invited lectures on bioanalytical method validation and bioequivalence and on pharmacokinetic and pharmacostatistical aspects of drug interactions, as well as chaired or co-chaired international meetings on these topics.

Raymond Gibson

Merck Research Laboratory, West Point, PA, USA

Dr. Gibson was a Senior Investigator (now retired) for Merck. He trained in organic chemistry and medicinal chemistry under the guidance of Dr. B.R. Baker at the University of California Santa Barbara (The Design and Synthesis of Inhibitors of the Enzyme Choline Acetyltransferase). He obtained an

NIH post-doctoral fellowship to work with Dr. R.D. O'Brien at Cornell University (Ithaca, NY) studying the nicotinic acetylcholine receptor and obtaining an exceptionally good understanding of ligand-receptor interactions. In 1976 he joined Dr. William Eckelman and Dr. Richard Reba at the George Washington University Medical Ctr., Department of Nuclear Medicine, in the early days of receptor-imaging. The goals were to develop single-photon emitting radiotracers for the β_1 -adrenoceptor in myocardium and radiotracers for imaging estradiol receptor in breast tumor metastases. Dr. Gibson initiated studies on the muscarinic receptor which eventually led to the development of 4- ^{123}I Iodo-QNB for imaging muscarinic receptors in the CNS, principally as a means of differential diagnosis of dementias, which led to clinical studies on muscarinic receptor concentrations in patients with dementias in collaboration with Dr. Dan Weinberger at NIMH. He joined Merck Research Laboratories in 1988. Dr. Gibson's efforts at MRL during the early years of the department were in the *in vitro* and *in vivo* characterization of new radiotracers for a variety of programs within the company. These studies led to development of radiotracers for the NMDA receptor, the angiotensin receptor (AT1: ^{123}I [Sar,Ile]-AngII, ^{123}I L-735,286, and ^{11}C L-159,884), endothelin receptor (ET_A: ^{123}I ET-1 and ^{11}C L-753,037), and CCK-A receptor (not published). He also conducted the critical studies which led to development of the NK1 receptor radiotracer (^{18}F SPARQ) and the CB1 receptor radiotracer [^{18}F]MK-9470). In addition to reported studies on mGluR5 radiotracers, Dr. Gibson collaborated with MRL scientists on the development of a radiotracer for the enzyme farnesyl transferase, the first reversible enzyme inhibitor used as a "site-specific radiotracer". In addition to efforts at MRL which also include the uses of microCT for drug development, he is a co-founder of the Society of Non-invasive Imaging in Drug Development (SNIDD) which became an institute of the Academy of Molecular Imaging in 2003. He has published 99 refereed papers, 21 book chapters, and contributed to editing two books.

Xiaochen Gu

Faculty of Pharmacy, University of Manitoba,
Winnipeg, MB, Canada

Dr. Xiaochen Gu obtained his degrees (B. Sc., Pharmacy, M. Sc., Pharmaceutics, Ph. D., Pharmaceutics, China Pharmaceutical University) in Nanjing, China, and came to Canada as a postdoctoral fellow in 1993. He joined the Faculty of Pharmacy, University of Manitoba after his postdoctoral training, and is now an Associate Professor of Pharmaceutical Sciences.

Dr. Gu's research interests include transdermal drug delivery, novel dosage form development and assessment, and controlled release techniques. In particular, his research in concurrent use of insect repellents and sunscreens has received greater interests from both the general public and the pharmaceutical industry. He has published over 100 research articles, and has been an invited speaker at numerous conferences and universities.

Dr. Gu was a recipient of New Investigator Grant in Oral Lipid-based Drug Delivery Systems by the American Association of Pharmaceutical Scientists. He is a member of Editorial Advisory Board of the Open Dermatology Journal, and reviews research papers and grants for numerous pharmaceutical journals and granting agencies. He teaches pharmaceutics and drug analysis in undergraduate pharmacy curriculum and supervises 4 graduate students at the University of Manitoba.

Urs Hafeli

Faculty of Pharmaceutical Sciences, University of
British Columbia, Vancouver, BC, Canada

Urs Hafeli is an assistant professor in the Faculty of Pharmaceutical Sciences at the University of British Columbia in Vancouver, Canada. Every two years, since 1996 he has organized and co-chaired the International Conference on the Scientific and Clinical Applications of Magnetic Carriers. At its last meeting in May 2008, the conference attracted more than 350 participants from 32 countries to Vancouver (for more information see www.magneticmicrosphere.com). Dr. Hafeli's research has concentrated on magnetic drug targeting with microspheres and nanospheres and on the preparation of biocompatible and biodegradable monosized particles. In order to make uniform particles, he is investigating flow focusing based on silica- and PDMS-nanotechnologies. The overall aim is to use drug releasing targeted particles for

radiopharmaceutical cancer therapy. Dr. Hafeli received a Pharmacy degree from the Federal Institute of Technology in Zurich, Switzerland and his Ph.D. from the Paul Scherrer Institute in Villigen, Switzerland. He spent 1.5 years as a postdoctoral fellow at the Joint Center of Radiation Therapy at Harvard University, followed by 11 years as a research scientist in the Radiation Oncology Department of the Cleveland Clinic Foundation.

Grier Harris

Appian International, Charlotte, NC, USA

[No bio provided]

Sui Yuen Eddie Hou

Depomed Inc., Menlo Park, CA, USA

Dr. Sui Yuen Eddie Hou joined Depomed in 2000 and is Senior Research Fellow, Formulation Science and Pharmacokinetics. Prior to Depomed, he was Group Leader, Formulation and Product Development at Penederm and Bertek Pharmaceuticals, a subsidiary of Mylan Laboratories. Before Penederm, he held technical and managerial positions at Advanced Polymer Systems. He obtained his B.S. in Pharmacy and Ph.D. in Pharmaceutics degrees from the University of Michigan, Ann Arbor and did postdoctoral research in the Department of Dermatology at the University of California, San Francisco. Dr. Hou has over fifteen years of experience in pharmaceutical product development in the areas of oral controlled release and topical dermatological products and has contributed to the development of six marketed drug products. He had been a co-investigator for an NIH Small Business Innovation Research grant. He also serves as an adjunct professor of pharmacy at the University of the Pacific, Stockton, California

Bev Incledon

Pacgen Biopharmaceuticals Corporation, Toronto,
ON, Canada

Dr. Incledon graduated from the University of Guelph with a Bachelors of Science degree in 1990 and started his scientific career as a protein chemist in the Immunology Department at the National Reference Laboratory for the Canadian Red Cross Society. From there he moved into the pharmaceutical industry as a biochemist in the drug discovery laboratories for Syntex Inc., where he

developed high throughput assays for HIV protease inhibitors. Following a few years in the industry Dr. Incledon returned to graduate school and received his Ph.D. in biophysics from the University of Guelph in 1998. Following Post Doctoral studies at Cornell University Dr. Incledon joined GlaxoWellcome as a senior scientist in analytical development and was promoted to group leader before moving on to Eli Lilly and Company. In 2001 Dr Incledon accepted an opportunity with Eli Lilly and Company to develop and lead a bioproduct development laboratory to support Eli Lilly and Company's synthetic peptide portfolio. Over the next 9 years Dr. Incledon grew the capabilities within the Eli Lilly's Canadian affiliate and was promoted to Director of the Lilly Analytical and Bioanalytical Research Laboratories. In this role Dr. Incledon was responsible for 21 development programs ranging from the pre-clinical to global submission stage. Currently Dr. Incledon is the Vice President of Research and Development for Pacgen Biopharmaceuticals Corporation, responsible for their portfolio management, research acquisitions, and all ongoing clinical programs.

Fakhreddin Jamali

Faculty of Pharmacy, University Of Alberta,
University of Alberta, Edmonton, AB, Canada

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 and served as the associate dean (1999-2004).

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 and toxicology of anti-rheumatic, analgesic and cardiovascular drugs. He also works on rapidly absorbed formulations.

He has published over 190 refereed articles, has been an invited speaker at many conferences, and has trained 30 PhDs. He is a principal investigator with the Centre of Excellence for Gastrointestinal Inflammation and Immunity Research and also a member of the Canadian Arthritis Network.

For his academic achievements and research, he has received the Canada's Killam Professorship and has been appointed is a Fellow of American

Assoc. Pharm. Sci. He is a recipient of the McKean Cattel Memorial Award of the American College of Clin. Pharmacol, the McCalla Professorship of the University of Alberta, the McNeil Award of Assoc Canadian Faculties of Pharm, Leadership Award of the Canadian Soc. Pharm. Sci. For his service to the public he has been honored with 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 has served as a member of the Health Canada's TPP Expert Advisory Committee on Bioavailability and Bioequivalence, and the Expert Advisory Panel on Nonsteroidal Anti-inflammatory Drugs. He is the founding president of Canadian Soc. Pharm. Sci., editor of J. Pharm. & Pharm. Sci. (www.cspCanada.org); has served as associate editor or editorial board of several journals. He teaches pharmacokinetics and is involved in pharmacy curriculum development.

Isadore (Izzy) Kanfer

Rhodes University, Grahamstown, South Africa

Prof. Kanfer (BSc (Pharm); BSc (Hons); PhD) was appointed to the Chair as the first Professor of Pharmaceutics at Rhodes University in 1980 and served as Head of Pharmacy at Rhodes University and Dean of the Faculty from 1999-2007. He was Visiting Professor in Pharmaceutics at the University of California, San Francisco (1980/81) and also at the University of North Carolina's School of Pharmacy in Chapel Hill(1990), in the USA. Prof. Kanfer spent several years in the Pharmaceutical Industry in Canada and served as the representative of the International Generic Pharmaceutical Alliances (IGPA) on the World Health Organization's (WHO) Committee on Multisource (generic) Pharmaceutical Products: Guidelines on registration requirements to establish interchangeability. He was appointed by the South African Minister of Health as a member of the South African Medicines Control Council (MCC) and served as Chairperson of the MCC's Expert Committee (Analytical) and also of the Complementary Medicines Committee. In addition, Prof Kanfer served as Vice-Chairperson of the MCC's Pharmaceutical and Analytical Committee which includes Bioavailability and Bioequivalence. He is a Founder Member and Past Chairman of the South African Academy of Pharmaceutical Sciences and was an inaugural member of the Editorial Advisory Board of the European Journal of

Pharmaceutical Sciences. He currently also serves as Associate Editor of the Journal of Pharmacy & Pharmaceutical Sciences and is a member of the Editorial Board of the Journal of Pharmaceutical & Biomedical Analysis. His main research interests involve the quality, absorption, safety and efficacy of medicines. He has contributed to over 100 research publications and conference presentations and is co-editor of 4 books in the series, *Generic Drug Product Development*. Professor Kanfer was the recipient of the Rhodes University Vice Chancellor's Distinguished Senior Research award for 2007 and was elected Emeritus Professor in 2008. He is an honorary life member of the South African Academy of Pharmaceutical Sciences and a Fellow of the South African Pharmaceutical Society. In 1998 he was elected to the Executive Committee of the Canadian Society of Pharmaceutical Sciences (CSPS) and has served as the Chairperson of the Heads of Pharmacy Schools Committee (South Africa) from 2000–2004.

Rav Kumar

Vice-President, R&D Operations, GlaxoSmithKline (GSK), Toronto, ON, Canada

Dr. Rav Kumar leads GSK's Canadian R&D organization of over 250 scientists and staff involved in global drug development, clinical trials and regulatory affairs. He is also a member of the Executive team which oversees the company's business in Canada.

Dr. Kumar is a pharmacy graduate with a PhD in pharmaceutical sciences for research into controlled delivery of drugs such as insulin, from the University of Bath in the UK.

He has more than 20 years of global drug development experience having worked a number of multinational pharmaceutical companies in the UK, France and North America.

Dr Kumar is President of the Canadian Society for Pharmaceutical Sciences which brings together government, academia and industry to improve drug research and development in Canada. He also serves as president of SAPNA – a voluntary organization dedicated to improving the health of South Asians in Canada. Dr Kumar has co-chaired the 2006 and 2007 Canadian Drug Information Association meetings and sits on the North American Advisory Council for DIA. He is a previous vice-chair of the Regulatory Affairs Committee at Rx&D. He was a member of the University of Toronto Advancement Board for the new Pharmacy building and Board

Governor at Hillfield Strathallan College in Hamilton.

Lorelei Lutter

Bio Pharma Services Inc. and ADA Medical Ltd., Toronto, ON, Canada

Ms. Lutter is Vice President of Business Development at Bio Pharma Services Inc., an FDA-inspected, Toronto-based CRO specializing in Phase I/IIa clinical trials and BE/BA/PK studies in healthy volunteers, special population, and patient populations. She is also VP of Business Development for ADA Medical Ltd., a research partnership group which has a growing network of 115 investigator sites in multiple therapeutic areas in Canada and US available for Phase IIb and Phase III clinical trials and clinical endpoint studies. Both Bio Pharma Services Inc. and ADA Medical Ltd. are physician-owned, and are headquartered in Canada, providing clinical trial support to pharmaceutical and biotech companies globally.

She has over 16 years of CRO and pharmaceutical industry experience, in both technical and business development areas. Previous to Bio Pharma and ADA Medical, Ms. Lutter held various senior business development, sales and marketing roles in several Canadian CROs including CANTEST Ltd. in Vancouver, and Biovail Contract Research and Pharma Medica Research in Toronto. She was also involved in outsourcing clinical trials at Genpharm in the scientific affairs department.

She has chaired two technical sessions to date – one at BIO in Toronto, and another at CSPS in Banff. She has also authored three articles to date, relating to clinical trials in Canada, spoken in several conferences relating to contract research services, and exhibited at numerous technical workshops and industry conferences in the last nine years.

Ms. Lutter has an Honours Bachelor of Science degree in Human Biology and Nutritional Sciences from the University of Toronto, and a Master of Business Administration degree from York University, Schulich School of Business. She is member of the American Association of Pharmaceutical Scientists, and she is currently Secretary of the Canadian Society for Pharmaceutical Sciences, and member of the organizing committee for this year's CSPS Symposium.

Mehul U. Mehta

Office of Clinical Pharmacology, CDER, FDA,
Silver Spring, MD, USA

Dr. Mehta is the Director, Division of Clinical Pharmacology I (DCP I), Office of Clinical Pharmacology, OTS, CDER (Center for Drug Evaluation and Research), FDA. His division is responsible for reviewing the clinical pharmacology (CP) and biopharmaceutics (B) aspects of the Cardio-Renal, Neuropharmacological and Psychiatric drug products. He received his B.Sc. and M. Sc. in Chemistry and Synthetic Organic Chemistry from the University of Bombay in 1976 and 1979, M.S. in Medicinal Chemistry from the University of Houston in 1981 and Ph.D. in Pharmacokinetics from the University of Pittsburgh in 1986. He joined the Division of Biopharmaceutics, FDA in 1986 as a reviewer, was promoted to the Section Head, Oncology and Pulmonary Drug Products, in 1992, to the Deputy Director, DCP I in 1995, and to the Director, DCP I in 1999. In addition to his review oversight, administrative, and management responsibilities, he currently co-chairs the CDER wide BCS (Biopharmaceutics Classification System) Committee, is the Sponsor of the OCP Working Group on Alcohol – Modified Release Product Interaction project and is a member of the CDER Biopharmaceutics Advisory Board. Past research interests have included Cyclosporine pharmacokinetics in liver transplant patients, anti-viral drug-drug interactions in monkeys, exposure based alternative to the maximum tolerated dose in carcinogenicity studies, and PK-PD considerations in the development of chemo-preventive agents. Current research interests include CDER Critical Path related research, e.g., disease progression modeling in Parkinson's disease, role of imaging in Neurological drugs and the possible extension of BCS. He is a member of several focus groups and planning committees in professional societies and is currently the Chair of the AAiPS (American Association of Indian Pharmaceutical Scientists) DC Metro Chapter. He has published numerous research papers and written several book chapters.

Bruce Meiklejohn

Eli Lilly and Co., Indianapolis, IN, USA

[No bio provided]

Eric Ormsby

Therapeutic Products Directorate, Health Canada,
Ottawa, ON, Canada

Eric has worked for Health Canada for 27 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.

Michael S Roberts

School of Pharmacy & Medical Sciences, University of South Australia, and School of Medicine, University of Queensland, Australia

Dr. Roberts (Bachelor of Pharmacy, University of Adelaide; MSc, PhD, DSc pharmaceutical science University of Sydney; MBA University of Queensland) was Professor and Chairman of Pharmacy at University of Otago, New Zealand from 1986-1989, when he took up a Research Chair in the School of Medicine at the University of Queensland. Recently, he has accepted an offer to become Professor of Therapeutics & Pharmaceutical Sciences, School of Pharmacy & Medical Sciences, University of South Australia. He has more than 300 peer reviewed research publications and has co-edited six research books. His key research areas of interest in rational drug design, drug delivery, pharmacokinetics and quality use of medicines are funded by the National Health & Medical Research Council, Australian Research Council and other bodies. In 2004, he was co-awarded the inaugural Australasian Pharmaceutical Science Association (APSA): APSA Achievement award "for outstanding achievements in pharmaceutical science" and in 2007 the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) biennial Michael Rand Medal for outstanding contribution to the disciplines of clinical and experimental pharmacology or

toxicology nationally and internationally. He has been active in developing pharmaceuticals and products, with a special interest in the skin, as evidenced by various patents, books, collaboration with pharmaceutical companies, international invitations to speak in this area and his active membership on a number of Australia's regulatory advisory committees. This work includes the application and development of various technologies to image and measure diseases, including the noninvasive examination of cells below the skin or other organ surface, and how diseases may be modified by pharmaceutical interventions. He also has a particular interest in facilitating beneficial patient outcomes through the translation of research into practice involving patients in both the hospital and community settings.

Amin Rostami-Hodjegan

Simcyp Limited, and The Medical School, University of Sheffield, Sheffield, UK

Amin Rostami is a "Professor of Systems Pharmacology" at the School of Medicine, University of Sheffield, and also the Director of Scientific R&D at Simcyp Limited (spin off company of the University of Sheffield). He obtained his PhD from the University of Sheffield in 1996 and joined the University of Sheffield as research assistant to Professor Geoff Tucker before progressing to Lecturer, Senior Lecturer, Reader and Full Professorship posts in 1997, 2002, 2005 and 2008, respectively. As the Director of Scientific R&D at Simcyp Limited, he leads a team of 20 scientists working on extrapolation of in vitro data on drug metabolism to predict in vivo pharmacokinetics and pharmacodynamics in virtual patient populations. His innovations in this field led to the receipt of the EUFAPS 2004 Award for "New Safe Medicines Faster" (jointly with Professor Geoff Tucker) and the OSCAR (Outstanding Scientific Contribution to Animal Replacement) award by Hadwen Trust for Humane Research in 2009.

Professor Rostami has been author/co-author of over 85 peer reviewed highly cited full articles (overall citation of >1200 and H factor of 19). He has been the elected Scientific Secretary of PKUK (the UK discussion group on PK) since 1998 and an elected member of EUFAPS Council and the Drug Metabolism Committee of IUPHAR. He also serves on the a number of Editorial Boards for journals related to clinical pharmacology and drug metabolism (including Br J Clin Pharmacol,

Biopharm Drug Dispos, Drug Metab & Pharmacokin, Curr Drug Metabolism, and Xenobiotica).

He has been an invited speaker at over 50 national and international meetings and led a number of workshops in the area of in vitro-in vivo extrapolation as applied to ADME in Drug Development.

Graham Ruecroft

Prosonix Ltd, Oxford, UK

Dr. Ruecroft (BSc (1983), MSc (1986) and PhD (1989) degrees in chemistry from Teesside Polytechnic, North London Polytechnic and the Open University (all UK) respectively) has held senior research and development positions in a number of biotechnology and pharmaceutical technology companies including Wellcome Foundation, Ferring Research, Chiroscience, ChiroTech, Dow Chemical and Ultrafine. In the 12 years spent at Chiroscience / ChiroTech / Dow Chemical he had responsibility for process development and manufacture of a range of chiral pharmaceutical products and is listed as inventor on a number of process patents in this area.

His key areas of interest include synthetic, physical organic chemistry, crystallisation science, and the use of ultrasound in industrial processing of pharmaceuticals. He joined Accentus C3 Technology (UK) as Head of Research and Development in 2003. He is a co-founder of Oxford based Prosonix, which bought the C3 Technology business in 2006, where he is now the Chief Technical Officer. He has responsibility for all chemistry research and development activities as well as external scientific liaison. He is author of a number of technical scientific papers and general reading articles. His more recent interests are involved with particle engineering of inhalable drug particles and he has responsibility for technical development and commercialization of some of the newer technology innovation within Prosonix for manufacture of crystalline particles for both oral and respiratory delivery. He is the named inventor on a number of particle engineering patents.

Dr Ruecroft is a member and chartered chemist of the Royal Society of Chemistry and has won awards from this institution in the area of technology innovation.

Frances J. Sharom

Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada

Dr. Sharom (BSc University of Guelph, Guelph, Canada; PhD Biochemistry, University of Western Ontario, London, Canada) is a Professor and Tier 1 Canada Research Chair in Membrane Protein Biology. She joined the faculty at the University of Guelph in 1980, where she was supported by an NSERC University Research Fellowship from 1980-1984. Dr. Sharom served as Director of the Guelph-Waterloo Graduate Program in Chemistry and Biochemistry (GWC²) from 1991-1994, and Director of the Biophysics Interdepartmental Group (BIG) Graduate Program from 2003-2008.

Her research interests lie in the field of membrane proteins in health and disease. Her laboratory is currently using various biophysical approaches (including fluorescence spectroscopy) to study the structure, function and interactions of two ABC transporters, namely mammalian P-glycoprotein (ABCB1) and bacterial MsbA, and the NPC1 protein, which plays an unknown role in intracellular cholesterol trafficking. The involvement of phospholipids, cholesterol and GPI-anchored proteins in lipid raft formation, and the interaction of PI-specific phospholipase with membrane surfaces is also under investigation. Dr. Sharom has published over 109 refereed papers, 10 book chapters, and 160 conference abstracts. She has been an invited speaker at many international conferences, and has trained 9 MSc and 14 PhD students.

Dr. Sharom has received several teaching awards, including an Ontario Confederation of University Faculty Associations (OCUFA) Teaching Award (1992), the Lieutenant Governor's Award for Teaching Excellence (1993), a University of Guelph Faculty Association Special Merit Professorial Teaching Award (1992), and the University of Guelph Faculty Association Distinguished Professor Award (2002). In 2006, she was designated the Jeanne Manery Fisher Award Lecturer of the Canadian Society of Biochemistry, Molecular & Cellular Biology (CSBMCB).

Dr. Sharom has been Vice-President, President, and Past-President of the Canadian Society of Biochemistry, Molecular & Cellular Biology (CSBMCB), and was Editor of the CSBMCB Bulletin from 2002-2008. She has served on grant review panels for the National Cancer Institute of Canada (NCIC), the Ontario Research

and Development Challenge Fund Medical Review Panel, and the Canadian Institutes of Health Research (CIHR), and is currently an Editor for Biochemical Journal.

Terrence L. Sills

Ontario Cancer Biomarker Network, Toronto, ON, Canada

Dr. Sills received his Ph.D. from University of Toronto in 1994, and was a Fogarty Visiting Fellow at the National Institute of Mental Health in Bethesda, MD until 1996. Following his tenure at the NIMH, Dr. Sills returned to Toronto where he was a NARSAD-funded Research Scientist at the Centre for Addiction and Mental Health.

Dr. Sills moved to industry in the spring of 1999, taking the position of clinical research scientist at Boehringer Ingelheim, where Dr. Sills worked on a number of international drug-development programmes (depression, stroke, female sexual dysfunction, and HIV/Aids). In 2003, Dr. Sills left Boehringer Ingelheim to co-found AXON Clinical Research, which provided clinical research consulting services to the biotech sector. At AXON, Dr. Sills worked on a number of development programs for small and mid-sized biotech companies.

Following the successful launch and growth of AXON Clinical Research, Dr. Sills joined the Ontario Cancer Biomarker Network as Vice President to develop and grow the organization into a profitable business, and to work towards applying biomarkers and diagnostics to the development of personalized therapeutics, the medical model that will become prevalent in the 21st century.

Randy Stroud

BioMS Technology Corp., Edmonton, AB, Canada

Randy Stroud, principal of Randy Stroud Consulting Inc., holds a science degree in chemistry from the University of Waterloo and has over 30 years of experience managing drug regulatory and technical projects in compliance with regulatory requirements. He has broad experience with Canadian and international drug regulatory systems including both pre-market registration and post-marketing compliance. He has held senior positions within Canadian pharmaceutical companies and has experience working internationally. His industry experience also includes many years working on a wide variety of issues, with colleagues, through a

variety of trade associations in the biotech, pharmaceutical, nutritional, natural health and medical device industries. He has chaired various committees and has served on boards of a variety of industry associations.

The dirucotide project started at the University of Alberta. Randy was asked by the University's Industrial Liaison Office to provide input into the project in the mid 1990's. The company BioMS was created in the late 1990's, and he was asked to help BioMS develop the drug to bring the promising therapy to patients with MS. From the late 1990's to present, Randy has been working on the dirucotide project, concentrating on the chemistry and manufacturing, preclinical, regulatory, and Quality Assurance requirements to support the clinical development programme for the drug. Past experiences with a wide variety of drug development and regulatory projects, as well as an international network of experts in the CMC, preclinical, regulatory, QA, and compliance areas were brought to the dirucotide project and allowed a successful development programme to be implemented. In 2007, BioMS partnered with Eli Lilly to continue the development of dirucotide for registration and commercial sale globally. For the past 1.5 years, Randy has been working cooperatively with dirucotide development teams at Eli Lilly to achieve registrations for dirucotide and make it available to patients with MS.

Bertrand Tavitian

Laboratoire d'Imagerie moléculaire expérimentale, CEA-Inserm, Orsay, France

Bertrand Tavitian (MD, Professor of Biochemistry) is head of the Laboratoire d'Imagerie moléculaire expérimentale (Experimental Molecular imaging Lab) at the CEA-Inserm in Orsay, France. He coordinates the European Network of excellence EMIL (European Laboratories in Molecular Imaging of Cancer) and the European Master in molecular imaging. His lab runs the platform for innovative technologies (PET, SPECT, CT and optical imaging) for small-animal imaging research.

His research focuses on in vivo techniques for molecular imaging of gene expression. He is involved in radiotracer development and validation and innovative instruments and methods for quantitative imaging in animals and Humans. He has a special interest in nucleic acids as biotechnological tools for molecular imaging.

Krishnan Tirunellai

Therapeutic Products Directorate, Health Canada, Ottawa, ON, Canada

Krishnan has Bachelor and Master degree in Pharmacy (India), and Ph.D. in Biopharmaceutics, (Dalhousie University, Canada). He served as a scientist at AstraZeneca and Patheon, in R&D and Manufacturing divisions for six years, and as a tenured Associate Professor, School of Pharmacy, Memorial University of Newfoundland, Canada, for seven years. For the past nine years Krishnan has been serving Health Canada in various capacities including Senior Reviewer, and Manager of the New Drugs Quality division. He is presently serving the Bureau of Pharmaceutical Sciences as a Senior Scientific Advisor.

Krishnan's areas of expertise are drug delivery systems and manufacturing and he has over 35 research papers and abstracts. He has served the ICH Q8(R) expert working group, and is presently serving the ICH – Q8, 9, 10 - Implementation Working Group.

Krishnan received Killam Scholarship for his graduate studies at Dalhousie. At Memorial University he received Bristol-Myers Squibb - Professor of the Year in 1995, and at Health Canada the first Claire Franklin Award for Excellence in Continuous Learning, in 2005 for his contributions in continuing education.

Yu Chung Tsang

Biopharmaceutics and Biostatistics, Apotex Inc., Toronto, ON, Canada

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 Steering Committee for the Bioequivalence Focus Group of the American Association of Pharmaceutical Scientists and the Bioequivalence Working Group of the European Generic Medicines Association.

Ricardo Vargas

Formulations and Processes, Purdue Pharma Canada, Pickering, ON, Canada

Ricardo (MSc, Industrial Pharmaceutical Sciences, University of Manchester, Manchester, U.K) is the Technical Manager Formulations and Processes at Purdue Pharma Canada. Ricardo has over ten years of experience in the pharmaceutical industry in both brand and generic companies. His areas of interests include Formulations Development of Immediate and Controlled release products, Technology Transfer, Process Optimization, Six Sigma methodology, PAT and the use of statistical analysis and experimental design.

He has several years of experience using Six Sigma methodology to improve manufacturing processes of solid dosage forms. He has also worked with and evaluated the feasibility of using different analytical methodologies such as FT Near Infra Red; Thermal Effusivity, and Near Infra - Chemical Imaging as PAT tools in research and manufacturing environments.

He got his Six Sigma Black Belt certificate in 2004 and contributed to the successful implementation of Six Sigma at the manufacturing facility of Pfizer Canada. He was recognized by Pfizer Global Manufacturing Americas area with the Create an Inclusive Environment 2004 Award. He was member of the Process Capability Global Design team as the representative from North America and the Pfizer Global Raw Material Change Evaluation Team. He led the Black Belt Community of Practise subchapter of East United States and Canada regions within Pfizer.

He has also been a key participant in the development, re-formulation, optimization and technology transfer of solid dosage forms at the pharmaceutical companies where He has worked. He led the implementation of the Product Launch department to optimize the technical transfer of Immediate Release and Controlled release solid dosage products at Apotex Inc.

The projects executed by Ricardo using his scientific knowledge in combination with his Six Sigma experience have permitted companies to

launch products that were considered not feasible and to improve several manufacturing processes by maximizing the efficiency, capacity and quality of products, which has represented millions of dollars in savings.

Bernard Vrijens

Pharmionics Research Centre, Visé, and University of Liège, Belgium.

Bernard Vrijens is Chief Scientist at the Pharmionics Research Centre, in Visé, Belgium and Adjunct Professor of Biostatistics at the University of Liège, Belgium. He did his graduate studies in the Department of Applied Mathematics and Informatics at the University of Ghent, Belgium, working on the development of biostatistical methods for estimating and analyzing variable exposure of humans to xenobiotics. He received his PhD in May 2002. His university education, concentrated in mathematics, was at the University of Liège (1992), and he received a Master's Degree in Biostatistics from the Belgian University of Limburg in 1993. Prior to his doctoral studies, he worked for Pfizer Corporation in Belgium and the US on biostatistical analysis of veterinary pharmaceutical products, from 1995-7. During his graduate studies, he was appointed to the Royal Commission that investigated the Belgian dioxin crisis, which had toppled the government and temporarily halted Belgian farm exports.

In a series of papers that commenced during his graduate studies, and are continuing, he has developed various ways of extracting clinical explanatory power from drug dosing histories, as ambulatory patients variably comply with prescribed drug dosing regimens. He started to build the Pharmionic Knowledge Centre (PKC®), the largest repository of data, publications, and technical documents related to electronically compiled dosing histories. This rich source of structured information constitutes the bases of an ongoing research program that allows his team to identify (a) the most common errors in dosing using a simple but robust taxonomy, (b) particular dosing errors that can jeopardize the efficacy of a drug (c) the optimal measurement-guided medication management program that can enhance patient compliance and maintain long term persistence. Co-author of 2 book chapters, over 30 peer reviewed scientific papers, and named as inventor on 2 patents.

Kishor M. Wasan

Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada

Dr. Kishor M. Wasan (B.Sc. (Pharmacy), Ph.D.) is a Distinguished University Scholar Professor & Chair of Pharmaceutics at the University of British Columbia in Vancouver, BC. In the 14 years that Dr. Wasan has been an independent researcher at UBC, he has published over 180 peer-reviewed articles and 210 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 was one of the recipients of the 1993 American Association of Pharmaceutical Scientists (AAPS) Graduate Student Awards for Excellence in Graduate Research in Drug Delivery, the 2001 AAPS New Investigator Award/Grant in Pharmaceutics and Pharmaceutics Technologies, the 2002 Association of Faculties of Pharmacy of Canada New Investigator Research Award and recently was named an AAPS fellow in 2006. In addition, Dr. Wasan was awarded a Canadian Institutes of Health Research University-Industry Research Chair in Pharmaceutical Development (2003-2008), was named a University Distinguished Scholar in April 2004 received the 2007 AAPS Award for Outstanding Research in Lipid-Based Drug Delivery and the 2008 AFPC-Pfizer Research Career Award. In April 2009 Dr. Wasan was named CIHR/iCo Therapeutics Research Chair in Drug Delivery for Neglected Global Diseases. Currently

Dr. Wasan's research is supported by several grants from The Canadian Institutes of Health Research, several pharmaceutical companies and the National Cancer Institute of Canada-Clinical Trials Group.

Jesse Zhu

Particle Technology Research Centre, and Department of Chemical and Biochemical Engineering, University of Western Ontario, London, ON, Canada

Dr. Jesse Zhu is a Professor and Canada Research Chair in the Department of Chemical and Biochemical Engineering, at the University of Western Ontario, in London, Canada. Dr. Zhu received his B. Eng. from Tsinghua University in Beijing in 1982 and PhD from the University of British Columbia in Vancouver in 1998, both in Chemical Engineering. After working briefly as a Research Scientist for Shell Research in the Netherlands, he started teaching in Canada in 1990. By 1999, he established the Particle Technology Research Centre at his university and has served as its Director until now. Dr. Zhu has also won many research awards including two of the three major awards given by the Canadian Society for Chemical Engineering. Prof. Zhu has been very active in fluid-particle research and particle technology, with over 300 publications and over a dozen of patents including two novel particle technologies for the pharmaceutical industry.

Poster Presentations
Day 1
Thursday, June 4, 2009

Day 1

Biomedical Sciences

1. Effect of Postprandial Hypertriglyceridemia on Pharmacokinetics of Clozapine and Norclozapine in Rats

Pavel Gershkovich¹, Ric Procyshyn¹, Alasdair M. Barr² and Kishor M. Wasan¹. ¹ Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia, Canada; ² Department of Anesthesiology, Pharmacology & Therapeutics, University of British Columbia, Vancouver, British Columbia, Canada.

Purpose: The aim of this study was to assess in the rat model whether the postprandial hypertriglyceridemia alters the pharmacokinetics of clozapine (a lipophilic atypical antipsychotic agent) and its metabolite, norclozapine, due to association with triglyceride rich lipoproteins.

Methods: Clozapine was administered as slow intravenous bolus via jugular cannula, at the dose of 4mg/kg (4mg/ml solution in PEG-400 50%, water 40%, ethanol 10% (v/v/v)). Severe hypertriglyceridemia was induced by oral administration of peanut oil (0.6ml) 6 h and 3 h before clozapine administration, at the time of clozapine administration and 3 h following clozapine administration. The mild hypertriglyceridemia group received the same doses of peanut oil except the 6 h before clozapine administration. Normolipidemic (control) animals received water instead of oil at the same time points. The blood samples were withdrawn via jugular vein cannula. The plasma concentrations of clozapine and norclozapine were determined by a means of HPLC. Plasma triglyceride levels were determined by enzymatic kit.

Results: The oral administration of peanut oil resulted in extremely significant elevation in area under curve of plasma triglyceride concentration-time profile ($AUC_{TG, 0-8h}$) in both severe and mild hypertriglyceridemia groups relatively to control animals (1566 ± 416 h*mg/dl, 883 ± 74 h*mg/dl, and 271 ± 28 h*mg/dl, respectively (mean $\pm$ SEM)).

A total clearance of clozapine from the plasma increased in both severe and mild hypertriglyceridemia states relatively to control animals (3161 ± 50 ml/h/kg, 3006 ± 273 ml/h/kg, and 2474 ± 101 ml/h/kg, respectively (mean $\pm$ SEM)). No other pharmacokinetic parameters of clozapine were significantly altered by postprandial hypertriglyceridemia. The levels of norclozapine obtained following intravenous bolus administration of clozapine to rats were very low and not different between the treatment groups.

Conclusions: Postprandial hypertriglyceridemia seems to affect the total clearance of clozapine in the rat model, probably due to association of this lipophilic drug with triglyceride-rich lipoproteins. Further studies are needed in order to explain the cause of clearance increase in hypertriglyceridemia states and to elucidate the relevance of this finding in clinical practice.

2. L-FABP Antioxidant Mechanism of Action

Jing Yan¹, Yuewen Gong^{1,2}, Yi-Min She⁴, Guqi Wang⁵, Michael S. Roberts⁶, and Frank Burczynski^{1,3}

¹Faculty of Pharmacy, University of Manitoba; ²Section of Hepatology, Department of Internal Medicine,

Department of Pharmacology and Therapeutics;

³Faculty of Medicine, University of Manitoba, Winnipeg, Canada; ⁴Centre for Biologics Research, Biologics and Genetic Therapies Directorate, Health Canada, Ottawa, Ontario K1A 0K9, Canada;

⁵McColl-Lockwood Laboratory, Cannon Research Center, Charlotte NC, USA 28232-2861;

⁶Department of Medicine, Princess Alexandra Hospital, University of Queensland, Woolloongabba, Queensland, Australia 4102.

Objective: Hepatocytes expressing liver fatty acid binding protein (L-FABP) are known to be more resistant to oxidative stress than those devoid of this protein. The mechanism for the observed antioxidant effect is not known. In this study we examined the antioxidant mechanism of recombinant rat L-FABP in the presence of a hydrophilic (AAPH) or lipophilic (AMVN) free radical generator.

Methods: Recombinant rat L-FABP was produced in *E. coli* and its amino acid sequence identified by MALDI QqTOF MS. Antioxidant activity was assayed using the thiobarbituric acid method.

Ascorbic acid served as a positive control for the AAPH system while α -tocopherol was used as a positive control for the AMVN system. L-FABP amino acid oxidative products were analyzed by MALDI-TOF MS.

Results: Recombinant L-FABP was observed to have better antioxidative activity when free radicals were generated in the hydrophilic system than in the lipophilic system. Oxidative modification of L-FABP included up to five methionine oxidative peptide products with a total of ~80Da mass shift compared to native L-FABP. Protection against lipid peroxidation of L-FABP after binding with palmitate or α -bromo-palmitate in AAPH or AMVN free radical generating systems indicated that ligand binding can partially block antioxidant activity.

Conclusion: We conclude that the mechanism of L-FABP antioxidant activity is through binding of free radicals by the methionine and cysteine amino acids. Moreover, exposure of the L-FABP binding site further promotes its antioxidant activity. In this manner L-FABP serves as a hepatocellular antioxidant and thus could prevent oxidative stress induced liver damage.

(This study was supported by the Canadian Institute of Health Research and the Australian National Health & Medical Research Council.)

3. Effect of Elevated Levels of Lipoproteins on the Electrocardiographic Effects of ($\pm$)-Halofantrine

Jigar P. Patel and Dion R. Brocks*. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada.

Purpose: To assess the influence of elevated plasma lipoproteins on the electrocardiographic (ECG) effects of ($\pm$)-halofantrine (HF) and desbutylhalofantrine (DHF) using an experimental rat model of hyperlipidemia.

Methods: Rats were rendered normolipidemic (NL) or hyperlipidemic (HL) on day one and day three with i.p injection of either saline or poloxamer 407 (1 g/kg), respectively. One day later, right jugular vein cannulation was performed. At 12 h after surgery, rats received either 4 doses of ($\pm$)-HF HCl (2, 5, 10, 15 or 20 mg dose) or vehicle for 2 d with 12 h interval between each dose. Measures of ECG were made from all groups of rats, recorded under light anesthesia, before i.p injection, before surgery, 12 h after 1st and 4th dose of HF. At 12 h after 1st dose of HF, blood sample was collected at the time

of ECG measurements. At 12 h after the last dose, rats were sacrificed to collect blood and heart tissues. Stereospecific HPLC was used to analyze HF and DHF enantiomers in plasma and heart. Commercially available assay kits were used to measure plasma triglyceride and total cholesterol concentrations.

Results: Vehicle had no significant effect on measured PR, RR, QT or QTc intervals. Plasma concentrations of both HF enantiomers were higher (~9 to 16 fold) in HL than NL rats, however for DHF only (-)-enantiomer concentration was higher (~3 fold) in HL than NL plasma. Compared to NL, no difference in heart concentrations of HF and DHF enantiomers was observed in HL rats. Unlike NL, plasma concentrations of HF enantiomers in HL gave no significant correlation with change in QTc interval. Similar results were observed when HF+DHF concentrations were examined vs. changes in QTc interval. Although in HL heart concentrations correlated significantly with both unbound plasma concentrations of HF enantiomers and plasma triglyceride concentrations, no correlation was observed with change in QTc interval. Similar results were observed with HF+DHF.

Conclusion: The HL state caused a weakening in the concentration vs. effect relationship of HF concentrations and QT interval prolongation.

Acknowledgement: Funded by CIHR MOP 87395.

4. The Effects of High Glucose Incubation on the RhoA/Rho Kinase Pathway in Cultured Cardiomyocytes

Anthony Gador, Hesham Soliman, Kathleen M. MacLeod. Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC.

Purpose: Diabetic cardiomyopathy is one of the major cardiovascular complications of diabetes leading to increased patient morbidity and mortality. Alarming 60,000 new cases of diabetes are reported yearly in Canada, yet despite extensive efforts the pathophysiology of diabetic cardiomyopathy remains incompletely understood. The monomeric GTPase, RhoA, and its downstream effector, Rho kinase (ROCK), have established signaling roles in cytoskeletal/contractile dynamics, hypertrophy and adhesion. Previous studies by this lab have linked over activity of the RhoA/ROCK pathway with diabetic cardiomyopathy and demonstrated that acute ROCK inhibition improves

heart contractile function in the streptozotocin diabetic rat model. Hyperglycemia, the principal finding in diabetes, has been found in other tissues to increase the production of reactive oxygen species (ROS). The relevance of this being, that elevated ROS have been implicated in the activation of RhoA. Determining if high glucose incubation activates the RhoA/ROCK pathway in cultured rat cardiomyocytes will provide further biochemical understanding of diabetic cardiomyopathy and may highlight future therapeutic targets for this currently untreatable complication.

Methods: Cardiomyocytes from adult male Wistar rats were isolated, made calcium tolerant and cultured on laminin. After an attachment period of 4 hours culture media was replaced with one containing normal glucose (5.5mM), high glucose (25 mM), or mannitol (19.5mM +5.5mM glucose). Cells were collected, lysed and processed at the following times: immediately after isolation, just prior to plating, 1h, 2h, 4h, 8h and 16h after plating. Cell lysates were prepared in Laemmli buffer and separated via SDS-PAGE. Gels were transferred to PVDF membrane, blocked in skim milk and immunoblotted for RhoA, ROCK I & II. Phospho-LIMK, a downstream ROCK target was used as an index of pathway activity. Signals were detected by chemiluminescence. Intracellular ROS production was assessed using the cell-permeable fluorescent probe dichlorofluorescein diacetate.

Results: Incubation of cardiomyocytes with high glucose resulted in a statistically significant increase in the expression of RhoA and ROCK I & II versus control incubations. Levels of phospho-LIMK were also increased suggesting RhoA/ROCK activation. Fluorescence microscopy showed increased intracellular ROS in cardiomyocytes incubated in high glucose conditions.

Conclusions: These findings suggest a relation between elevated RhoA/ROCK expression and activity with high glucose conditions. This study, along with other investigations, may elucidate how the diabetic state leads to cardiomyopathy through RhoA/ROCK dysregulation.

5. Regulation of P-Glycoprotein Expression by the Viral Envelope Protein gp120 and Pro-Inflammatory Cytokines in Human Glial Cells

Tamima Ashraf , Patrick T. Ronaldson, and Reina Bendayan. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada.

Purpose: P-glycoprotein (P-gp) is an efflux pump that exports anti-HIV drugs and reduces the bioavailability of antiretroviral drugs at several sites including the brain. Previously, we demonstrated that gp120 induces pro-inflammatory cytokine secretion (TNF- α , IL-1 β and IL-6) and reduces P-gp protein expression in primary cultures of rat astrocytes, a cellular reservoir of HIV-1 virus. However, whether P-gp is regulated in a similar way in human astrocytes is unknown at this stage. This study aims to recognize possible mechanisms of cytokine secretion and determine the regulation of P-gp by gp120, pro-inflammatory cytokines and signaling pathways in human astrocytes.

Methods: Primary cultures of human fetal astrocytes (HFAs) were treated with gp120 (1.0nM), for 6 and 24 hours in the presence or absence of SN50 (1 μ M; NF- κ B inhibitor), CXCR4 and CCR5 neutralizing antibodies (1mg/ml). TNF- α , IL-1 β and IL-6 secretion in response to gp120 were measured using ELISA analysis. Astrocytes were also treated with 0.5ng/ml and 10 ng/ml concentrations of TNF- α or IL-6 for the desired time (6h, 12h and 24h). In addition, IL-6 treatments were also performed in the presence of SN50. Immunoblot analysis was subsequently used to determine the protein expression of CXCR4, CCR5 and P-gp. Functional assay with [3 H]digoxin was performed to determine P-gp activity in gp120 treated cells.

Results: Immunoblot analysis confirmed the presence of CXCR4 and CCR5 receptors in HFAs. However, pretreatment of the cells with only CCR5 neutralizing antibody attenuated the TNF- α , IL-1 β and IL-6 secretion. Treatment with gp120 reduced the protein expression and increased cellular accumulation of [3 H]digoxin. IL-6 treatment decreased P-gp protein expression whereas TNF- α treatment increased its protein expression. However, P-gp protein expression was not altered when cultured cells were exposed to gp120 or IL-6 in the presence of SN50, an inhibitor of NF- κ B.

Conclusion: Our data show that the interaction of gp120 with CXCR4 and CCR5 receptors are

necessary for cytokine secretion and also demonstrate that the regulation of P-gp protein expression by gp120 and cytokines are NF- κ B pathway dependent in human astrocytes.

Supported by the Canadian Institute of Health Research (CIHR) and the Ontario HIV treatment network (OHTN)

6. Regulation of P-Glycoprotein (P-gp) by Orphan Nuclear Receptors in Human Brain

Gary N.Y. Chan and Reina Bendayan. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.

Purpose: Pregnane X Receptor (PXR/SXR), Constitutive Androstane Receptor (CAR) and Peroxisome Proliferator-activated Receptor Gamma (PPAR γ) are orphan nuclear receptors that have been recognized as species-specific xenosensors which regulate membrane transporters (P-gp, MRPs, ABCG2) and metabolic enzymes (cytochrome P-450s). Previous studies performed in the liver and intestine have suggested that the expression of MDR1 (P-gp) is transcriptionally regulated by PXR or CAR. However, evidence demonstrating the direct molecular interaction between PXR or CAR and MDR1 gene in the brain is presently lacking. Our study aims to investigate the role of PXR and CAR in the regulation of P-gp expression at the BBB and in glial cells (i.e., astrocytes).

Methods: An immortalized human brain microvessel endothelial cell line (hCMEC/D3) and a primary culture of human fetal astrocytes were used in this study. Immunoblotting, immunofluorescence and immunogold cytochemistry studies at the electron microscope were performed to examine protein expression and cellular localization of P-gp, CAR and PXR. In vitro transfection of PXR small interfering RNA (siRNA) in hCMEC/D3 cells was used to down-regulate basal PXR protein level.

Results: Expression of P-gp, PXR and CAR in hCMEC/D3 cells was confirmed by immunoblotting and immunocytochemistry in hCMEC/D3 cells. Treatment of these cells for 72h with 10 μ M of human PXR ligands (ritonavir, SR12813 and rifampin) resulted in 2 to 5-fold increase in P-gp expression. This effect was significantly attenuated in PXR-siRNA transfected cells. Nuclear translocation of PXR in the presence of SR12813 was observed using immunofluorescence. Treatment of fetal astrocytes with SR12813 compound as well

as treatment of hCMEC/D3 cells and fetal astrocytes with human CAR ligand (CITCO) is presently investigated in our laboratory.

Conclusion: These data suggest that orphan nuclear receptors are involved in the regulation of P-gp expression in human brain microvessel endothelial and glial cells. Since several drugs are known ligands of these receptors, their inductive properties on P-gp expression may contribute to further restrict the permeability of several drugs to their brain target site.

Supported by the Canadian Institutes of Health Research.

7. Multivariate Analysis of Audible Acoustic Emissions from High-Shear Wet Granulation

Erin Hansuld,¹ Lauren Briens,¹ Joe McCann,² Aryn Sayani². ¹The University of Western Ontario, Faculty of Engineering, London, ON, Canada; ²GlaxoSmithKline Canada Inc., Pharmaceutical Development, Mississauga, ON, Canada.

Purpose: Audible acoustic emissions have shown potential for monitoring high-shear wet granulation and detecting end-point. The objective of the research was to evaluate the use of multivariate analysis to classify wavelet or fast Fourier filtered acoustic signals as wetting or end-point.

Methods: Acoustic data was collected at 40 000 Hz for three formulations using a condenser microphone positioned inside the air exhaust of a PMA-10 Niro-Fielder high-shear granulator. The signals were down-sampled to 1000 Hz and wavelet or fast Fourier transform (FFT) analysis was applied in 10s segments. A Partial Least Squares Discriminant Analysis (PLS-DA) modeling approach was then used to classify the data as wetting or end-point; where end-point was defined based on formulator expertise and substantiated with analyses of granule size, flowability, and tablet hardness.

Results: The acoustic signals from granulations of three formulations were filtered using wavelet or FFT analyses and classified by PLS-DA. The wavelet analysis was carried out to five levels using three different wavelets (daubechies 4, coiflet 5 and symlet 2). Preliminary analysis showed the first level of details was sufficient for classification. The model fit (R^2) and predictive ability (Q^2) were further optimized by eliminating coefficients with low contribution (<0.03) or variable importance (<1). The final PLS-DA models were able to distinguish between wetting and end-point for all

three wavelets with the best distinction between groups achieved using the coiflet 5 wavelet (Figure 1). PLS-DA analysis of the FFT signal was performed using all frequency components from 20-1000 Hz. The model was improved by eliminating frequency components with low contribution (<0.03) or variable importance (<1), resulting in a reduced variable model that could distinguish end-point from wetting (Figure 2).

Fig. 1. PLS-DA scores plot from coiflet 5 detail 1

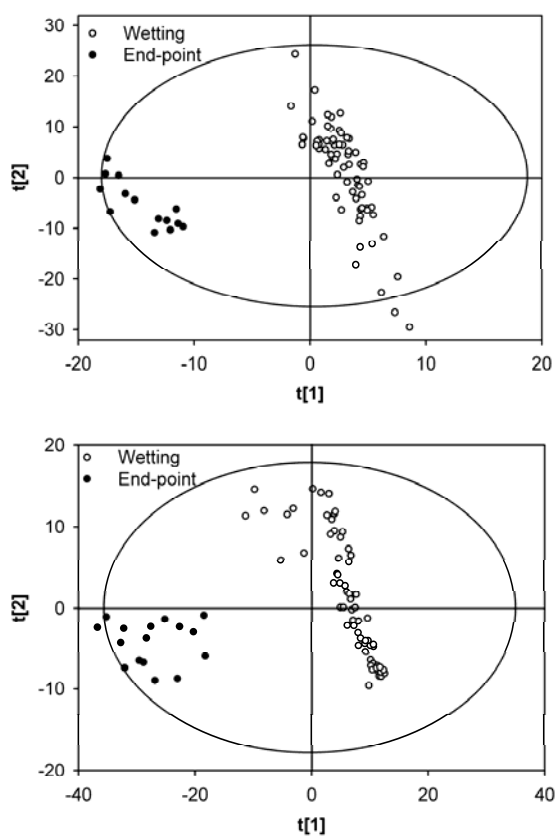


Fig. 2. PLS-DA scores plot from frequency wavelet coefficients for formulation 1 components for formulation 1

Conclusions: For three formulations the results show it is possible to use a PLS-DA multivariate modeling approach to classify granulation end-point from wavelet or FFT processed audible acoustic emissions with cumulative Q^2 values that support use of the models for prediction.

8. Glucosamine Prevents Adjuvant Arthritis and Down-Regulation of Calcium Channel Receptors caused by inflammation

Mohammadhossein Gilzad Kohan, Fakhreddin Jamali. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

Purpose: Calcium channel blockers including verapamil, affect the heart by prolonging PR interval. However, this effect is reduced by inflammation (i.e. rheumatoid arthritis and myocardial infarction), even in the presence of elevated plasma drug concentration. Control of inflammation and administration of HMG-CoA reductase inhibitors, angiotensin II receptor blockers (ARBs), and infliximab restore the diminishing effect of inflammation on cardiac receptors. Glucosamine is a naturally occurring amino sugar, which is believed to have the property to slow down and, perhaps, reverse inflammatory process.

Purpose: Using a rat model of adjuvant arthritis, we investigated the role of glucosamine in restoring the reduced response to verapamil in presence of inflammation. In addition, the effect of glucosamine on the development of arthritis was investigated.

Methods: Adult male Sprague-Dawley rats were randomly assigned to four groups ($n=6$): inflamed-treated, inflamed-placebo, control-treated, and control-placebo. On day zero, 0.2 ml Mycobacterium Butyricum in Squalene (50 mg/mL) or saline was injected to the inflamed and control groups, respectively. The treated groups were administered glucosamine hydrochloride (300 mg/kg/day, p.o. commenced on day zero), while the control groups received saline. On day 14, ECG leads were implanted s.c., a single oral dose of 25 mg/kg verapamil administered, and PR interval measured in certain time points. On day 17, animals were cannulated in the jugular vein and after recovery, the same verapamil dose administered again and serial blood sample collected. Blood samples were analyzed for verapamil using HPLC. Nitric oxide (NO) plasma concentration was assessed indirectly by measuring the concentrations of its stable products nitrite and nitrate. During the experiment, animal's paw diameter and weight were monitored.

Results: All rats that received Mycobacterium butyricum but not treated with glucosamine developed arthritis. Glucosamine treatment

completely prevented arthritis. As expected, inflamed-placebo rats had up to 4 fold greater plasma verapamil concentration and demonstrated reduced response to verapamil in terms of PR prolongation. Treatment with glucosamine of the inflamed rats prevented any reduction in response to verapamil and increased verapamil concentrations. Serum nitrite concentration was significantly elevated by inflammation but not in the glucosamine- treated inflamed rats.

Conclusion: Glucosamine prevent adjuvant arthritis and down-regulation of calcium channel receptors. If extrapolated to humans, our data may present a safe and effective alternative approach to prevention of cardiovascular problems associate with arthritis.

9. Expression of ABC Drug Efflux Transporters in a HIV-1 Transgenic Rat Model

Kevin Robillard and Reina Bendayan. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.

Purpose: ATP-binding cassette (ABC) membrane-associated drug efflux transporters, P-glycoprotein (P-gp) and multidrug resistance associated protein 1 (MRP1), can significantly restrict the permeability of antiretrovirals into target tissues leading to sub-therapeutic drug concentrations and the formation of viral reservoirs such as the brain and testes. Our previous studies demonstrated that HIV-1 viral envelope glycoprotein-120 (gp120) can trigger an inflammatory and oxidative stress response which can alter the expression of these transporters in primary cultures of rat astrocytes. The objectives of this study are to investigate, *in vivo*, the effect of HIV-1 viral proteins on the functional expression of P-gp and Mrp1 in a non-infectious HIV-1 transgenic rat model.

Methods: We used a non-infectious HIV-1 Sprague-Dawley rat model (HIV-rat) containing a *gag/pol*-deleted provirus which express 7/9 HIV-1 genes. Tissues and serum were isolated from two month old male HIV-rat or age-matched wild-type control rats (WT-rat). Tissues were analyzed for P-gp and Mrp1 mRNA expression using real-time quantitative PCR (qPCR). ABC protein expression in tissue lysates was determined using antibody specific-immunoblots. Serum levels of pro-inflammatory cytokines (i.e., TNF- α and IL-6) were analyzed using a specific enzyme-linked absorbent assays (ELISA).

Results: Although serum levels of TNF- α increased 2-fold in the HIV-rat group (30 pg/ml) compared to the WT-rat group (<15 pg/ml), IL-6 levels were found similar in the two groups. No difference was shown in P-gp mRNA expression in brain, kidney, liver, heart and testes by qPCR. In contrast, HIV-rat Mrp1 relative mRNA expression was found to be modestly but significantly decreased in brain (0.63 fold) and heart (0.59 fold) and significantly increased in testes (1.50 fold) when compared to the WT-rats. Immunoblotting data in HIV-rat brain lysates demonstrated a significant decrease in P-gp expression (5.9-fold) when compared to WT-rats.

Conclusions: The observed increases in the serum TNF- α level in HIV-rats suggest the presence of an inflammatory mediated response. Overall, it appears that gene and protein expression of ABC drug efflux transporters is altered in this HIV rodent model. In particular, P-gp expression in the HIV-rat brain tissue was significantly decreased. These data corroborate our previous *in vitro* findings in glial cells triggered with gp120 where we observed a significant decrease in the functional expression of P-gp. Further studies need to be performed in this rodent model to confirm that changes in the expression of transport proteins are related to circulating levels of HIV viral proteins and/or an inflammatory response.

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10. Pharmacokinetics of Maloxicam Administered as Regular and Fast Dissolving Formulations to the Rat: Influence of Gastrointestinal Dysfunction on the Bioavailability and Bioequivalence

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

Purpose: It is believed that acute pain suppresses *nervus vagus*, thereby, influences gastrointestinal (GI) secretion and motility, the two factors that are necessary for disintegration and dissolution of solid dosage forms. We studied the pharmacokinetics of meloxicam and the effect of GI dysfunction on the oral bioavailability and bioequivalence using a marketed (Brand) and a fast dissolving (FD) formulation.

Methods: In simulated gastric juice, FD was disintegrated in 30 s and released 30% of its

meloxicam in 15 min and 60% in 2 h. Brand disintegrated in 4.5 min with dissolution rate of 5.6% in 30 min that stayed plateau for the 2 h experiment time. To reduce GI motility and secretion (dysfunction) we suppressed the vagus nerve by intraperitoneal injection of 20 mg/kg propantheline 1 and 2 h before meloxicam administration. Meloxicam (0.9 mg/kg) was administered to both healthy control and vagally suppressed rats i.v. (n=4-6/group) as well as orally in a paired random fashion as crushed Brand or FD tablets (n=7/ group). Serial (0-48 h) blood samples were collected for pharmacokinetic and bioavailability studies.

Results: Systemic pharmacokinetics of meloxicam was not affected by vagal suppression. Absolute bioavailability of meloxicam, based on 0-48 h measurement, was close to 1 regardless of the type of formulation and treatment. Vagal suppression, however, significantly reduced AUC_{0-24} ($\mu\text{g}\cdot\text{h}\cdot\text{mL}^{-1}$) for Brand (control, 58.8 ± 22.0 vs treated, 22.1 ± 9.7) but not for FD (control, 63.5 ± 17.9 vs treated, 64.6 ± 8.9) indicating reduced absorption rate for the former. The peak time for Brand was also significantly delayed by over 20 h for Brand and not for FD. Under the control condition, bioequivalence was confirmed between FD and Brand but not in the vagally suppressed rats, indicating a condition-dependent bioequivalence.

Conclusion: The effect of gastric dysfunction that is reported during pain episodes on the drug absorption can be obviated if the disintegration and dissolution become independent of gastrointestinal motility and secretion. If extrapolated to human bioequivalence studies, our data suggest that bioequivalence data generated under healthy conditions may not necessary reflect parity under disease condition.

11. Gbetagamma Subunits of Heterotrimeric G Proteins Regulate Cell Adhesion Through Rap1a and its Effector Radil

Syed Mukhtar Ahmed, Stephane Angers. Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario.

Introduction: G-protein coupled receptors (GPCRs) are the largest family of integral membrane proteins capable of transducing extracellular cues into cellular responses. GPCRs transduce the information provided by a diverse assortment of extracellular stimuli via a family of heterotrimeric G-proteins and have been implicated in virtually all cellular

processes. Agonist binding promotes the GPCRs to adopt an active conformation leading to the activation and dissociation of $G\alpha$ and $G\beta\gamma$ subunits of G-proteins. While much has been studied about the nature and the functions of $G\alpha$ effectors, little is known about $G\beta\gamma$ -mediated cellular signalling.

Methods and Results: In an effort to identify novel effectors of the $G\beta\gamma$ subunits, we isolated $G\beta$ protein complexes from cultured cells using tandem affinity purification and analyzed their compositions by mass spectrometry. Validating our approach, several $G\alpha$, $G\gamma$ subunits, which are known interactors of $G\beta\gamma$ proteins were detected. Interestingly, several peptides corresponding to the previously uncharacterized protein Radil were also identified in the $G\beta$ complex. Reciprocal purification of the Radil protein complex confirmed the interaction of this protein with $G\beta\gamma$ proteins and identified Rap1a. These results suggest that Radil may be a protein interfacing $G\beta\gamma$ and Rap1a signalling. Further affinity purification and protein localization experiments indicated that Radil is recruited to the plasma membrane upon activation of Rap1a. We also found that the interaction of Radil with $G\beta\gamma$ depends on the activation state of Rap1a, as its inactivation with expression of Rap1GAPII can completely inhibit binding to both Rap1a and $G\beta\gamma$. Since most described functions of Rap1a are related to cell adhesion we hypothesized that the Rap1a/Radil/ $G\beta\gamma$ complex functions to regulate cell adhesion. Overexpression of $G\beta\gamma$ and Radil both lead to increased human fibrosarcoma cells' (HT1080) adhesion to fibronectin (FN) matrix, comparable to what is seen upon overexpression of a constitutively active (Rap1Q63E) Rap1 mutant. The increased cell adhesion in response to $G\beta\gamma$ or Radil overexpression was reversed by Rap1GAPII, implying that the modulation of cell adhesion to fibronectin matrix by these factors is Rap1a dependent. Second, endogenous stimulation of the fMLP GPCR strongly upregulated cell adhesion in a $G\beta\gamma$ and Rap1-dependent manner. Over-expression of dominant negatives of $G\beta\gamma$ (α -transducin or Grk2-ct) partially rescued adhesion mediated by $G\beta\gamma$ overexpression or fMLP.

Conclusions: The regulation of cell adhesion through GPCRs is known to be important in different contexts such as leukocytes biology and cancer cells metastasis. However, the molecular mechanisms that play downstream of GPCR activation to regulate adhesion are poorly understood. Our study shows that $G\beta\gamma$ play a previously unrecognized role for this process and

unveils the function of the new G β γ /Radil/Rap1 protein complex in cell adhesion signalling.

12. Impact of VEGF₁₆₅b Sialylation Level and IFN α 2b O-glycosylation on their Pharmacokinetic Behaviors in Rats

Fatemeh Afkhami^{1, 2}, Satya Prakash², Yves Durocher^{1*}. ¹ Biotechnology Research Institute, National Research Council Canada, Montreal, Quebec, Canada; ² McGill University, Montreal, Quebec, Canada.

Purpose: IFN α and VEGF₁₆₅b are angiogenesis inhibitors with potential application in tumor suppression. However treatment with these molecules may require frequent dosing if they are rapidly cleared from the circulation. This study aims to evaluate the impact of VEGF₁₆₅b hypersialylation and IFN α O-glycosylation on their pharmacokinetic properties.

Methods: O-glycosylation site of IFN α was abolished by site-directed mutagenesis. Both wild-type and mutated IFN α were expressed in HEK293 cells. VEGF₁₆₅b was also produced by transfection of HEK293 cells with or without a plasmid encoding a sialyltransferase. The quantity of sialic acid in purified VEGF samples was determined. Furthermore the pharmacokinetic parameters of purified VEGF₁₆₅b, hypersialylated VEGF₁₆₅b, mutated IFN α and wild-type IFN α were evaluated following the injection in rats.

Results: Higher level of sialic acid was found in the presence of sialyltransferase (VEGF-SIAT). No significant differences in the half-life of mutated and wild type IFN α or VEGF₁₆₅b vs. VEGF-SIAT were found.

Conclusion: These results suggest that 1) overexpression of sialyltransferase efficiently increases sialylation level in recombinant proteins; 2) O-glycosylated IFN α does not have better pharmacokinetic profile in rat vs. a non-glycosylated mutant. Whether these results can predict their behavior in primates or humans is however unknown.

13. A High-Throughput Screening Assay to Identify Novel Transporters and Proteins that Regulate Glucocorticoid Signalling

Lilia Magomedova and Carolyn L. Cummins. Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.

Purpose: Glucocorticoids (GCs) are steroid hormones that mediate their physiological effects through binding to the intracellular glucocorticoid receptor (GR). The central dogma in endocrine signalling is that due to lipophilicity, GCs enter the cell by passive diffusion without any selectivity. Several lines of evidence suggest this is not true. 1) GC influx kinetics are different from that of simple diffusion. 2) Studies performed using hepatocyte membrane vesicles show that GC uptake can be inhibited. 3) P-glycoprotein (P-gp) has been shown to actively efflux GCs in the brain, demonstrating that these lipophilic molecules utilize transporters to cross the plasma membrane. The purpose of this study is to develop a functional screening assay capable of detecting changes in ligand driven GR activation to identify novel GC-interacting proteins (such as a GC importer) from a human cDNA library.

Methods: HEK293 cells were transiently transfected using calcium phosphate with a one-hybrid assay system that includes the yeast GAL4-DNA binding domain fused to the GR ligand binding domain and the GAL4 upstream activator sequence luciferase reporter. To determine the ability of the screening assay to detect changes in either the GR activation or ligand concentration, HEK293 cells were cotransfected with a number of known GR interacting proteins (doses from 0.15ng-15ng) which include the corepressors RIP140 and SHP, the coactivator TIF2, and the efflux pump, P-gp. Cortisol was added at a sub-saturating dose. A trial screen for GR activating proteins from the human cDNA library was performed in a 96-well plate format using pools of ~50 cDNAs/well.

Results: Addition of 300nM cortisol to HEK293 cells in the presence of GAL4-GR and the luciferase reporter resulted in a >18-fold increase in luciferase activity compared to background. Cotransfection with the corepressors, RIP140 and SHP showed a dose-dependent repression of GR activity. A significant increase in GR activity was observed in the presence of the coactivator TIF2 (5ng) and a significant decrease in GR activation was observed in the presence of P-gp (5ng). A preliminary screen

of a cDNA library identified 3 positive hits (>2-fold) out of 85 wells screened.

Conclusion: We have validated the sensitivity of the high-throughput cotransfection assay for detecting significant differences in GC levels and/or GR activity. Moreover, a preliminary screen of a human cDNA library demonstrated that the assay is capable of detecting positive signals, which will be further screened to identify novel GC or GR interacting proteins and transporters.

14. Insulin Glargine Safety in Pregnancy: A Transplacental Transfer Study

Erika K. Pollex^{1,2}, Denise Feig^{1,3}, Angelika Lubetsky², Gideon Koren^{1,2}. ¹The University of Toronto, Toronto, ON; ² The Division of Clinical Pharmacology, Hospital for Sick Children, Toronto, ON, and the Division of Endocrinology and Metabolism, Mount Sinai Hospital, Toronto, ON³.

Purpose: Gestational diabetes mellitus is a common medical complication in pregnancy. Untreated, hyperglycemia associated with gestational diabetes can lead to numerous obstetric complications. Treatment has been shown to reduce the rate of perinatal complications, thus stressing the need to determine the safest and most effective method of treatment. While insulin remains as the gold standard therapy, the potential for maternal hypoglycemia with insulin injection has led to the development of newer insulin analogs. Insulin glargine is a human insulin analog that is longer lasting and has a smooth time-action profile and no pronounced peak when compared to currently used NPH insulin. The degree of transfer of insulin glargine across the placenta has not yet been determined. The objective of this study is to determine the extent of transfer of insulin glargine across the human placenta.

Methods: The placental transfer of insulin glargine was investigated using the ex vivo dual perfusion system of isolated human placental lobules. Placentae were obtained following cesarean section delivery of non-complicated term pregnancies. In a closed circuit experiment, insulin glargine was added at therapeutic concentrations of 150pmol/L as well as concentrations 1000 fold higher to the maternal perfusate. Samples of perfusate were taken from the maternal and fetal circuits during pre-control, experimental, and post-control periods for measurement of insulin glargine and markers of tissue viability.

Results: Following perfusions completed in the presence of therapeutic maternal levels of insulin glargine, there does not appear to be transfer across the placenta (n=5). Insulin levels were not detectable in the fetal circuit. However, following perfusions in the presence of very high levels of insulin glargine (1000-fold higher), levels in the fetal circuit were detectable and increased over the course of the perfusions (n=4). The rate of appearance into the fetal circuit was 100 fold lower than the rate of disappearance from the maternal circuit, 0.57 ± 0.09 and 42.05 ± 17.82 pmol/min/g tissue respectively.

Conclusion: Pharmacotherapy in pregnancy involves an increasing number of conditions and drugs with fetotoxic potential. Identification of drugs that do not cross the placenta is critical to being able to provide the best treatment options for the mother while protecting the unborn. Insulin glargine, at therapeutic concentration, does not appear to cross the placenta at detectable levels. Data obtained from all concentrations tested suggest that the placenta may be metabolizing or sequestering the drug in the tissue.

15. Can Teas Inhibit Cytochrome P450 3A4?

Tam, T.W.¹, Liu, R.¹, Arnason, J.T.¹, Foster, B.C.^{1,2}. ¹ Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, Ottawa, ON; ² Therapeutic Products Directorate, Health Canada, Ottawa, ON

Purpose: This study was conducted to determine if teas commonly used as beverages or traditional medicine have the potential to affect the metabolism of therapeutic products by inhibiting the drug-metabolizing enzyme cytochrome P450 3A4 (CYP3A4).

Methods: Water extracts were prepared from commercially available teas and some wild crafted sources. These samples included blends of herbal, black, or green teas. Goji (*Lycium barbarum*) tea, commonly used as Traditional Chinese Medicine was prepared from fresh and dried berries. Tea (AD01) made from a medicinal plant commonly used by First Nations, was prepared from dried leaves. Samples of the teas were tested for their inhibition effects on recombinantly expressed human CYP3A4 using a microtiter fluorometric assay.

Results: The black teas had the strongest inhibitory effect on CYP3A4. The green teas moderately inhibited CYP3A4, and the herbal teas had a weak to moderate effect. None of the commercially available

teas showed the potential to inhibit CYP3A4 through mechanism-based inhibition. Goji tea had weak inhibition towards CYP3A4, and the effect was stronger with the tea prepared from dried berries. AD01 tea showed a progressive increasing inhibition towards CYP3A4 the longer the leaves were brewed.

Conclusions: Teas prepared as beverages or traditional medicine have the potential to inhibit CYP3A4 and may affect the metabolism of concomitantly used therapeutic products such as drugs or natural health products. In particular, black teas and highly concentrated AD01 tea has the greatest potential to affect drug metabolism, especially when they are consumed frequently and in large amounts. Further studies are warranted to determine if these effects are clinically significant.

16. Enhanced Wound Healing in Diabetic Rats with a Sustained Nitric Oxide Delivery System

Yan Li, Ping I. Lee. Faculty of Pharmacy, University of Toronto, Toronto, Ontario.

Purpose: Local delivery of nitric oxide has shown potential clinical utility in treating wound infection with topical applied NO gas. Several studies have linked impaired wound healing in diabetic ulcer patients to NO deficiencies at the wound site. Exogenous NO supplementation has been shown to be beneficial for the healing of diabetic ulcers in animals. However, this mode of treatment is limited by the short half-life of NO and the intrinsic instability of available NO donors. On the other hand, S-nitrosoglutathione (GSNO), an endogenous nitrosothiol, has emerged as a desirable NO donor for wound healing. However, since free GSNO is both thermally and photolytically labile, the release of NO from existing GSNO based system can not be maintained for more than several hours. This study evaluates the effect of localized and extended delivery of NO from a GSNO-based polymeric complex on wound healing associated with diabetes.

Methods: 15 Male Sprague-Dawley rats were injected intraperitoneally with streptozotocin (60 mg per kg body-weight in citrate buffer 0.1 mol/L, pH 4.5) to induce diabetes. A full thickness excisional wound was created on each diabetic rat by removal of the skin and panniculus carnosus using a 8 mm biopsy punch. For each animal group, either GSNO attached or blank polymeric complex powders were applied to the wound with the assistance of a few drops of sterile saline.

Afterwards, on day 0, 4, 7, 10, 13 and 16 after wounding, photographs of the wound sites were recorded using a digital camera, the surface area of each lesion was quantified using Image-Pro Plus 5.0 software and plotted as a function of time.

Results: Preliminary *in vivo* testing in diabetic rat model demonstrates that a single topical application of this sustained release NO delivery system can effectively accelerate wound closure ($p < 0.05$). The apparent wound condition in terms of open area and granulation tissue appears to be much better in the test group as compared with the control group. There is a statistically significant difference in wound closure tendency between the control and test group.

Conclusion: The results suggest that GSNO immobilized polymeric complex system hold considerable promise for wound treatment particularly for chronic non-healing wounds such as diabetic ulcers. As demonstrated, this system is able to produce a consistent, sustained and biologically effective release of NO on wound area, which offers advantages over currently available topical NO donors.

Drug Delivery and Pharmaceutical Technology

17. Development of a Discriminating Dissolution Method for a Poorly Soluble Drug Product Using a Cyclodextrin Based Media

Le Hoang, Peter Coles, Betty Kan, Lydia Labenda, Geoffrey Lynch. GlaxoSmithKline, Mississauga, Ontario, Canada.

Purpose: To develop a dissolution method, for a poorly soluble active, that would encompass the principles of Quality by Design (QbD) such that the dissolution assay would be capable of discriminating the impact of changing manufacturing process parameters on the performance of the drug product.

Method: A number of surfactants and/or dissolving agents were evaluated as part of the dissolution media in order to obtain release profiles that could discriminate differences in the manufacturing process for a BSC class IV drug substance with very low aqueous solubility and poor permeability. The dissolution was studied at pH 4 in order to approximate the pH of the stomach in the fed state in which the drug product was evaluated in a clinical

study.

Results: The commonly used surfactants such as CTAB (cationic surfactant), sodium dodecyl sulphate (ionic surfactant) and poloxamers (non ionic surfactants) were found to be unsuitable as they either would interact with the drug substance (SDS) or were not capable of providing sink conditions (CTAB). Although poloxamers were able to provide sink conditions, the rate of dissolution of the drug substance in the poloxamer containing media was too slow to provide meaningful dissolution profiles.

Hydroxypropyl β cyclodextrin, an uncommonly used dissolving agent for dissolution methods and capable of forming an inclusion complex with the drug substance, (technical grade, low cost), was found to be capable of producing discriminating profiles for the drug product when evaluated against numerous manufacturing process differences.

Conclusion: The dissolution rate of the active compound in the cyclodextrin media was significantly improved. The cyclodextrin was able to provide sink condition for a wide range of the drug product strengths without compromising the discriminatory characteristics. The discriminatory power of the cyclodextrin based dissolution media was clearly demonstrated when evaluated using samples from a large granulation/compression based design of experiment (DoE) study.

18. Femtosecond Laser Ablation/Fragmentation in Aqueous Medium: An Efficient Route for the Production of Drug Nanocrystals

Sukhdeep Kenth¹, M. Meunier² and J-C. Leroux^{1,3}.

¹Canada Research Chair in Drug Delivery, Faculty of Pharmacy, Université de Montréal, P.O. Box 6128, H3C 3J7 Montreal, Qc, Canada; ²Department of Engineering Physics, École Polytechnique de Montréal, P.O. Box 6079, H3C 3A7, Qc, Canada; ³Institute of Pharmaceutical Sciences, ETHZ, 8093 Zürich, Switzerland.

Purpose: This project investigates the fabrication of paclitaxel nanoparticles using femtosecond laser ablation and fragmentation in aqueous medium, which has recently proven to be effective to produce fine inorganic nanoparticles with narrow size distribution¹.

Methods: The laser experiments were conducted using a Ti: sapphire laser providing 120 fs pulses centered at 800 nm (1 kHz repetition rate). The paclitaxel tablet was placed in a glass beaker

containing poloxamer 188 solution. With the beam focused on the tablet, the power (25 - 400 mW) and treatment time (10- 60 min) were varied (Figure 1a). For the fragmentation treatment, the colloidal paclitaxel suspension was transferred to a glass vial, and the laser was focused in the stirring suspension for 60 minutes from 50 to 400 mW (Figure 1b). Size distribution of the nanocrystals was measured by dynamic light scattering. Drug content and chemical degradation of the nanocrystals were evaluated by HPLC.

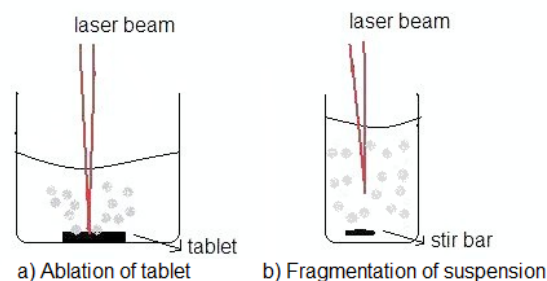


Figure 1. Schematic representation of ablation and fragmentation methods.

Results: Evaluation of the ablation process demonstrated that increasing power from 25 to 400 mW, resulted in a higher concentration of the paclitaxel nanocrystals in suspension, indicating a greater ablation of the tablet. Particle size was independent of power since particles ranging from 800-1500 nm were observed at all powers. The optimal ablation condition was selected as: treatment of 20 min and power of 150 mW. The second-step fragmentation process was evaluated from 50-400 mW which refined particle size down to 100-500 nm, however significant degradation was observed. As the overall efficiency of the two step ablation/fragmentation process was limited by the amount of drug ablated in the initial step, an alternative strategy based upon fragmentation alone was explored. Paclitaxel suspensions (prepared by adding drug powder to poloxamer solution) were fragmented from 50-400 mW. As the power increased, smaller nanocrystals were obtained, however higher degradation was observed. Presently, various strategies are being established to decrease degradation.

Conclusion: A distinct method has been developed in order to fabricate paclitaxel nanocrystals. The successful fulfillment of this project may translate into improved methods for the preparation of drug nanoparticles.

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19. An Injectable Chitosan-Phospholipid Blend for Sustained and Localized Delivery of Docetaxel

Payam Zahedi, Raquel De Souza, Micheline Piquette-Miller and Christine Allen. Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada.

Purpose: Sustained and localized delivery of chemotherapeutics presents a “magic bullet” effect for cancer therapy by providing high drug concentrations at the tumor site, extended drug exposure and reduced systemic toxicity. In the present study, an injectable chitosan-phospholipid blend is put forth as a strategy to achieve sustained and localized delivery of docetaxel, an antimetabolic agent with considerable activity in the treatment of breast, lung and ovarian cancers, following intraperitoneal administration.

Methods: The physico-chemical and rheological properties and *in vivo* performance of the injectable chitosan-phospholipid blend were evaluated. The stability properties of the blend were assessed by turbidity and pH measurements. Molecular interactions within the blend were characterized using FTIR spectroscopy and confocal laser scanning microscopy. Degree of injectability was measured by attaining viscosity profile as a function of shear stress for the blend. *In vitro* docetaxel release profile from the blend was evaluated in physiologically relevant media. The biodistribution of docetaxel in plasma and relevant tissues was evaluated following intraperitoneal injection of the blend in healthy CD-1 mice. Finally, hepatotoxicity and systemic inflammation were assessed by alanine aminotransferase activity in serum and by measuring levels of circulating Interleukin-6, respectively.

Results: The stability of the blend was confirmed *in vitro* by turbidity measurements and attributed to specific molecular interactions and the organization of the materials within the blend, as evidenced by FTIR analysis and confocal laser scanning microscopy, respectively. The chitosan and phospholipid were found to colocalize in regions surrounding a mean object area of 11.2 μm^2 with colocalization coefficients of 43% and 46% for the polymer and lipid, respectively. The chitosan-phospholipid blend was shown to provide a reliable barrier that minimized initial burst release of

docetaxel and afforded sustained drug release, both *in vitro* ($2.7\% \pm 0.2$ per day) and *in vivo* ($4.4\% \pm 0.7$ per day). Constant concentrations of docetaxel were observed over a two week period in plasma and relevant peritoneal tissues, with no signs of toxicity or inflammation, following intraperitoneal administration in healthy CD-1 mice.

Conclusion: This localized delivery system has excellent potential for treatment of cancers, such as ovarian, that reside in the peritoneal cavity.

20. Effect of Administration of Milk on the Bioavailability of Diclofenac in Beagle Dogs

Fahad I. Al-Jenoobi^{*1}, Ibrahim M. El-Bagory¹, Mohsen A. Bayomi¹, Aws Alshamsan², Mohammed M. El-Dardiri¹, Mohammed Al-Sinady¹. ¹Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia; ²Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

Introduction: Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) that is used mainly to reduce inflammation and control pain. The most important side effect of diclofenac that affects patient compliance is gastrointestinal complaints. Many patients are recommended to receive an ulcer-protective drug as prophylaxis during long-term treatment and the drug is also recommended to be taken orally with food or milk to avoid stomach upset. This study has investigated the effect of concomitant administration of cow milk on diclofenac bioavailability.

Methods: Five healthy male beagle dogs of mean weight 10.8kg were used. Each dog administered one tablet of 50mg diclofenac potassium (Cataflam®) with either 200ml water, 200ml full cream milk or aqueous solution containing 5.12g sodium casinate. A washout period of 2-weeks was ensured between different phases. Venous blood samples (5ml) were taken from the femoral vein into heparinized tubes before drug administration and at selected time intervals up to 10 hr. Blood samples were assayed for diclofenac content using a modified HPLC method. Dissolution study was carried out for diclofenac 50 mg immediate-release commercial tablets (Cataflam®) in either 500 ml distilled water or 500 ml aqueous calcium chloride solution containing the amount of 226 mg calcium similar to that present in 200 ml cow milk. The dissolution apparatus (USP II) with 50 rpm paddle

rotational speed was used in the study at 37 ± 0.5 °C. Drug dissolution was monitored with respect to time for 60 min.

Results: The oral administration of cow milk with Cataflam® (immediate-release 50 mg diclofenac potassium tablet) to beagle dogs significantly decreased the values of C_{max} and rate of absorption of diclofenac (by about 40%) while, T_{max} and extent of drug absorption were not significantly altered. In an attempt to investigate the causes for the decrease in diclofenac absorption rate in presence of milk, the effect of oral administration of casein (as caseinate) on the pharmacokinetic of diclofenac potassium was also investigated in dogs. Administration of caseinate was found to significantly affect T_{max} and rate of absorption (C_{max}/UAC_{∞}) which showed a little but significant decrease compared with control. The expected increase of diclofenac dissolution, combined with the effect of presence of 200ml solution containing 5 gm casein in stomach that may delay gastric emptying rate ended up with a significant delay (11.9%) in drug absorption. The effect of calcium ions on dissolution of diclofenac potassium was also investigated. Calcium ions dramatically affect the dissolution of diclofenac and it was concluded that calcium salt of diclofenac that might be initially formed caused slower dissolution rate than that of potassium salt.

Conclusion: It could be concluded that although the administration of diclofenac with milk can help in controlling stomach upset which is considered an important side effect of the drug, oral administration of milk with diclofenac can significantly decrease diclofenac bioavailability and accordingly the efficiency of the drug due to both the effect of calcium on diclofenac dissolution in addition to delay in gastric emptying rate.

21. Bladder Tissue Uptake of Paclitaxel-Loaded Nanoparticulate Formulations for use in Intravesical Bladder Cancer Therapy

Antonia T. Tsallas¹, John Jackson¹, Helen M. Burt¹.
¹Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada.

Purpose: Current drug delivery methods for the treatment of bladder cancer are in part limited by poor drug penetration into the bladder wall. The present work describes the use of paclitaxel loaded nanoparticulate formulations for use in intravesical therapy of superficial bladder cancer.

Methods: Paclitaxel loaded methoxypolyethylene

glycol-block-poly(D,L-lactic acid) (MePEG-PDLLA) micelles were prepared by a solvent evaporation technique. The FDA approved formulation, Taxol® was used as a control. Both formulations were doped with radioactive paclitaxel. Approximately, 2 cm diameter pieces of freshly excised pig bladder were mounted in a diffusion cell and treated with paclitaxel for 120 min. Tissue was then sectioned into 60 µm slices and paclitaxel concentration was determined in sequential sections.

Results: The average total bladder tissue concentrations of paclitaxel from MePEG-PDLLA and Cremophor EL® micelles were 34.9 µg/g and 7.5 µg/g, respectively. Tissue concentrations then declined exponentially with respect to tissue depth. Approximately, 0.4% of the initial dose was recovered in bladder tissues treated with paclitaxel loaded MePEG-PDLLA micelles and 0.1% of the initial dose was recovered in bladder tissues treated with paclitaxel loaded Cremophor EL® micelles. The amount of paclitaxel that penetrated into the bladder tissue was significantly higher from MePEG-PDLLA loaded micelles than from Cremophor EL® micelles.

Conclusion: Paclitaxel was taken up in effective amounts by bladder tissues. MePEG-PDLLA loaded micelles provide an improved method to delivering paclitaxel to bladder tissue as compared to the Cremophor EL® micellar formulation (Taxol®). This effect may arise from MePEG-linked adherence of the polymer formulation to the mucous membrane of the bladder or excessive sequestration of paclitaxel in the Cremophor EL® micelles. Paclitaxel represents a suitable drug candidate for intravesical bladder cancer therapy because of its high lipophilicity and tissue penetration characteristics.

22. Novel Buccoadhesive Bilayered Films of Insulin-Formulation and *In vitro* Evaluation

Jasjeet Kaur Sahni, Farhan.J.Ahmad, and Roop.K.Khar. Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi, India.

Purpose: Buccal delivery has been preferred for the delivery of peptides and proteins as compared to other transmucosal routes due to its inherent advantages. In the present study an attempt was made to formulate and evaluate buccoadhesive bilayered films for the systemic delivery of insulin. The bilayered films comprised of an immediate

release effervescent film aimed at providing immediate relief to diabetic patients and a controlled release film aimed at maintaining the basal serum insulin levels essential for therapeutic effect throughout the period of application.

Methods: 1. Formulation of bilayered buccal films. The buccal films were prepared by solvent casting technique. The controlled release buccal film layer containing 50IU/patch human insulin was prepared by using CP 934 in combination with different film forming polymers and water as solvent system. Buccoadhesive effervescence based immediate release films containing 20IU/patch human insulin were prepared by using HPC-M, a mixture of sodium bicarbonate and monosodium citrate in different ratios (1:1,1:2 and 2:1) and anhydrous ethanol (15 ml). Propylene glycol was used as the plasticizer in both the films. The immediate release film was then attached to the sustained release buccoadhesive film mechanically by using anhydrous ethanol. In order to ascertain unidirectional release of the drug a backing layer of ethyl cellulose was formulated and adhered to the bilayered formulation.

2. *In vitro* evaluation of buccal bilayered films. The bilayered buccal films were evaluated *in vitro* for physical characteristics, folding endurance, surface pH, bioadhesive strength, *in vitro* dissolution and permeation across bovine mucosa.

Results: The bilayered films formed were complete, homogeneous, flexible, non-sticky and smooth. They exhibited a surface pH 7.16 ± 0.06 , folding endurance >250 , bioadhesive strength and adhesion time of 17.69 ± 0.26 g and 480 minutes respectively. The optimized formulation exhibited an initial burst release of 28.51% within 15 minutes followed by a total 98.79% drug release in a sustained manner over a period of 8 hours, which was the desired time of application. This release pattern is beneficial for patients who require insulin immediately. Maximum permeation of 20.01% drug was observed from the optimized formulation over a period of 8 hours after incorporating sodium deoxycholate as a permeation enhancer.

Conclusions: Buccoadhesive bilayered films were successfully prepared and characterized *in vitro*. The optimized formulation had satisfactory physical and mechanical characteristics, surface pH, bioadhesive strength, *in vitro* release, bioadhesive strength and permeation through the bovine mucosa.

23. Enhancement of Docetaxel Solubility via Conjugation of Formulation-compatible Moieties

Loan Huynh,[†] Jean-Christophe Leroux,[‡] Christine Allen*,[†]. [†] Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada; [‡] Institute of Pharmaceutical Sciences, ETHZ, Zürich, Switzerland; ^{*} Faculty of Pharmacy, University of Montreal, Montreal, QC, Canada.

Purpose: The delivery of hydrophobic drugs is plagued by low drug loading levels (i.e. low drug to material ratio) and poor drug retention in the delivery system following administration. In order to address these challenges, this study focused on optimizing the compatibility between the drug and the excipient that forms the core of the delivery system.

Methods: Computer-based theoretical calculations were employed to direct the design of docetaxel conjugates with enhanced solubility in the internal phase of a nano-emulsion formulation. The theoretically-identified optimal docetaxel conjugates were synthesized by direct attachment of lauroyl moieties through an ester linkage to docetaxel.

Results: In comparison to docetaxel, the conjugates exhibited significantly improved solubility in oil as predicted by our theoretical calculations. This contributed to high drug entrapment efficiencies (up to 97%) and a high drug loading capacity (5.7 % w/w) for the docetaxel conjugates. The mono-substitution of an acyl group at C-2' of docetaxel resulted in a conjugate with thirty-seven- to forty-six-fold lower cytotoxicity than that of the parent drug in two human cancer cell lines. Importantly, the activity exerted by the mono-substituted docetaxel on the cancer cells was due in part to the cytotoxicity of the parent drug that was released via hydrolysis of the conjugate under biologically relevant conditions. In contrast, di- and tri-substitution of acyl groups at C-2', C-7 and/or C-10 of docetaxel resulted in non-hydrolysable conjugates which were found to be inactive.

Conclusion: Our results show that computer-based theoretical calculation followed by experimental validation of solubility, lipophilicity and cytotoxicity is a promising strategy for enhancing drug-material compatibility while ensuring that the activity of the parent drug is not abolished. The mono-substituted docetaxel species was identified as the prodrug with the requisite properties for future consideration.

24. Formulation Reverse Polymeric Micelles for Pharmaceutical Applications

Marie-Christine Jones, Hui Gao, Jean-Christophe Leroux. Canada research Chair in Drug Delivery, Faculty of Pharmacy, Université de Montréal, Montreal, Quebec, Canada.

Purpose: To assess the ability of reverse polymeric micelles to act as solubilisers for peptides in non-aqueous organic media and improve their activity following subcutaneous or oral administration.

Methods: Star-shaped (4- to 8-arms) and linear poly(glycidyl methacrylate)s were synthesized by atom transfer radical polymerization as precursors of poly(glycerol methacrylate)s (PGOHMA)s.

The hydrophilic PGOHMA backbones were rendered amphiphilic through the esterification of pendant hydroxyl functions with acyl chlorides (12 to 18 carbons). The formation of micellar aggregates in non-aqueous media was confirmed by interfacial tension measurements. Particle size was determined by dynamic light scattering (DLS). Partition studies were conducted to evaluate the ability of RPMs to improve the solubility of peptides/proteins in oil. Different formulations were then prepared using vasopressin as a model peptide. The impact of emulsification on the release kinetics of the encapsulated peptide was studied *in vitro*. The antidiuretic effect of vasopressin (30 µg/kg) was assessed following subcutaneous and oral administration to rats.

Results: Alkylated PGOHMAs were shown to self-assemble into 20-60 nm reverse micelles (RMs) in organic solvents and/or oil. These nanosized aggregates were able to reversibly extract anionic dyes from water and solubilise them in an organic phase. In addition, the encapsulation of vasopressin in RMs greatly improved its solubility in ethyl oleate, an oily vehicle. This particularity led to the evaluation of RM for the formulation of vasopressin in non-aqueous organic media. *In vitro*, release studies showed that the entrapped peptide diffused slowly out of an oily RM solution (<15% in 7 days). In contrast, the release rate was significantly augmented upon emulsification of the formulation (50-70% in 8 h). *In vivo*, the oral administration of vasopressin encapsulated in RMs led to an improved pharmacological activity when C18-modified polymers were employed. The latter formulation was also injected subcutaneously to rats, where it produced a sustained antidiuretic effect (>48 h vs 8-10 h for the control solution).

Conclusion Star-shaped alkylated PGOHMAs were synthesized from multi-functional initiators. These amphiphilic polymers were shown to self-assemble into RMs in organic, apolar media above a threshold concentration. RMs acted as potent solubilizers, substantially increasing the affinity of hydrophilic dyes and peptides for the organic phase. This property was exploited in the elaboration of a non-aqueous vasopressin formulation which demonstrated increased therapeutic activity following oral and subcutaneous administration to rats.

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25. Investigations into the Physicochemical and Dissolution Characteristics of Solid Dispersions of Active Pharmaceutical Ingredients

Ranajoy Majumdar ^[1], Kenneth Alexander ^[1]. ^[1] Division of Industrial Pharmacy, Department of Pharmacy Practice, College of Pharmacy, The University of Toledo, Toledo, Ohio USA.

Purpose: The formulation of solid dispersions is a promising approach to enhance the dissolution rates and hence the bioavailability of poorly water-soluble drugs. However the key limitations had been processing the solid dispersions into dosage forms and reversion of the high energy amorphous form of drug to the lower energy crystalline state on storage. This study proposes to formulate ternary solid dispersion granules avoiding the limitations and further to investigate the physicochemical and dissolution characteristics of the solid dispersion granules after preparation and upon storage for four weeks at 40°C/75% RH.

Methods: Ternary solid dispersion granules were prepared by Hot-melt granulation in which the drug, Ibuprofen was dispersed in a molten dispersion carrier and coated onto an adsorbent. Polyethylene Glycol (PEG) 8000[®] and Gelucire 50/13[®] were used as the dispersion carriers and Neusilin US2[®] (magnesium aluminosilicate) was used as the surface adsorbent. We have investigated the physicochemical and dissolution characteristics of the drug at different times with the aid of various complimentary techniques such as DSC, XRD, FTIR and UV spectrometry.

Results: Dissolution rate of Ibuprofen was slightly enhanced from the granules in some formulations upon storage for 2 and 4 weeks. The mechanism for

increase in drug dissolution from the solid dispersion granules is investigated and is supposed to involve hydrogen bonding between the drug and Neusilin. The XRD patterns clearly indicated amorphous character of the granules after preparation and also indicated an increase in degree of amorphous character after storage for some formulations. FTIR scans were indicative of hydrogen bonding between the drug and Neusilin.

Conclusion: The further increase in drug dissolution on storage involves two competing phenomena of conversion of crystalline drug to amorphous hydrogen bonded state on storage that seems to increase the drug dissolution whereas the phenomenon of Ostwald ripening decreases the drug dissolution on storage. The solubility of drug in the dispersion carrier is the factor that determines the predominant mechanism by governing the flux towards the surface of Neusilin. Our studies have shown that Ibuprofen has moderate solubility in both Gelucire and PEG and both the competing mechanisms plays a moderate role in determining the solubility of the dispersion granules on storage. Overall this study proposes a formulation technique to increase the solubility and bioavailability of poorly soluble API's that have moderate to good solubility in the dispersion carrier, avoiding the limitations of traditional solid dispersions.

26. Co-Encapsulation of Doxorubicin and Mitomycin C in Polymer-Lipid Hybrid Nanoparticles Enhances Anticancer Synergy in Multidrug Resistant Human Breast Tumor Cells

Adam J. Shuhendler¹, Richard Cheung¹, Janet Manias¹, Allegra Connor¹, Andrew M. Rauth², Xiao Yu Wu¹. ¹Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada; ²Ontario Cancer Institute, Toronto, Ontario, Canada.

Purpose: Cancer chemotherapy can fail due to drug resistant phenotypes (MDR) within heterogeneous tumor cell populations. We previously demonstrated the anticancer synergy between doxorubicin (DOX), a substrate of MDR proteins, and mitomycin C (MMC), a second-line anticancer drug, and delineated the mechanism of the synergy. Based on findings that the synergistic interaction of DOX-MMC takes place in the nuclei, new solid polymer-lipid hybrid nanoparticles (PLN) co-loaded with DOX and MMC (DM-PLN) were formulated and their efficacy and mechanism of overcoming MDR

in cancer cells were investigated.

Methods: The DM-PLN were prepared by mixing melted myristate, PEO-40-sterate, and PEO-100-sterate with aqueous Pluronic F68, DOX, MMC, and an anionic polymer, followed by ultrasonication and cooling in cold water. The encapsulation efficiency and drug loading were determined spectrophotometrically. Drug release from DM-PLN was determined in pH 7.4 buffer at 37°C. The anticancer efficacy of the DM-PLN was examined in human breast cancer drug resistant MDA435/LCC6MDR1 cells by clonogenic assay and compared with that in wild type MDA435/LCC6 cells. Median Effect Analysis (MEA) was performed to characterize the synergy of DOX and MMC. Cellular uptake and intracellular trafficking of the PLN were determined by confocal fluorescence microscopy with fluoresceinamine isomer I-labeled lipid, Hoescht 33342 stained the nuclei and Vybrant DiI stained cell membranes. The cell death mechanism was studied by Titer-TACS TUNEL assay and immunocytochemistry. DNA double strand breaks (DSB) were detected using anti- γ H2AX antibodies, and apoptosis using anti-activated Caspase 3 antibodies. A Cellomics VTi imager was used to visualize immunocytochemistry.

Results: The MEA confirmed that DOX and MMC act synergistically in the human breast tumor cells. Co-encapsulation of DOX and MMC circumvented MDR completely with the same cell kill percent in both the MDR and wild type cells and reduced the effective dose up to 30-fold as compared to free drug solutions. The PLN were taken up by cells through membrane-bound vesicles and then trafficked to the perinuclear region of the cell in both wild type and MDR cells. The immunocytochemistry images revealed that DM- PLN induced DSB prior to apoptosis supporting the mechanism of synergy of DOX and MMC proposed.

Conclusions: By integrating the anti-tumor synergy of DOX and MMC with our PLN drug delivery platform, we have developed a new nanoparticle formulation that is able to effectively overcome MDR in human breast cancer cells.

27. An investigation into the Hindered Settling of Pumice Using Various Surfactants

Shikha Rath^[1], Kenneth Alexander^[2]. ^[2] Division of Industrial Pharmacy, Department of Pharmacy Practice, College of Pharmacy, The University of Toledo, Toledo, Ohio USA.

Purpose: The particle size analysis of drug substances is an important concept for pre-formulation development. In order to make a stable suspension formulation and to improve the sedimentation rate becomes a very challenging experience. It is difficult to disperse the particles in a medium without the occurrence of sedimentation. The purpose of this study is to demonstrate how one can retard the sedimentation rate using various surfactants in a mixture of Glycerin and Water as the dispersion medium.

Methods: The sedimentation study was performed, using surfactants as the dispersing agents. It was observed that the size and charge on the surfactants was directly dependent on the molecular weight of the dispersing agent and the charging system. Linear plots were observed using the mono-exponential model equations from Richardson and Zaki, Steinour and Dollimore-McBride theories. Any bi-exponential models were investigated and the results thus obtained for the aggregate particle size for pumice was anticipated to be less than 300 μm . The present study involves evaluating various particle sizes of pumice determined by sieving, sedimentation, Laser Diffraction Technique and Microscopy. DSC procedures were performed to evaluate the presence of bound water.

Results: The sedimentation rate for Pumice was slightly reduced when using Tween 20, Benzalkonium chloride and Sodium stearate and it was further reduced by the solvent used (Glycerin and Water). Particles which are retained on each sieve were further examined using microscopy. The highest percentage of Pumice retained occurred on mesh (#300). The particle size obtained was between 44 microns and 53 microns.

Conclusion: The mean value for the results obtained for dry pumice dispersed in an aqueous media determined by various methods was anticipated to be approximately 30 μm . The range of values obtained by sieving was 25-88 μm . Microscopic evaluation of particle shape is a reliable method to determine the morphology of the particle. A low-temperature DSC procedure was performed to evaluate the presence of bound water. The experiment was performed on both

the dispersed and non-dispersed systems and the percent relative bound water established for the three surfactants.

28. Formulation and Characterization of Mixtures of Psycho-Active Drugs with their Excipients

Satya Girish Avula^[1], Kenneth Alexander^[4], Alan T. Riga^[2]. ^[4] Division of Industrial Pharmacy, Department of Pharmacy Practice, College of Pharmacy, The University of Toledo, Toledo, OH, USA; ^[2]Department of Chemistry, Cleveland State University, Cleveland, OH, USA.

Purpose: Poor water solubility and slow dissolution into the gastrointestinal tract (GIT) are the two major obstacles impeding the pharmaceutical industry in releasing new dosage forms into the market. These issues have been responsible for the rejection of 70% of the potentially active drugs discovered. The solution to this obstacle is formulating the drugs with known and accepted excipients, which may involve the formation of eutectics. Appropriate mixtures have played a key role in improving the absorption of many compounds by increasing their solubility and dissolution properties. Solving this problem within the pharmaceutical industry will lead to the release of new dosage forms to the market. This study proposes to formulate mixtures of bipolar drugs (e.g. Nortriptyline and Quetiapine fumarate) with excipients (e.g. PEG, Acetyl salicylic acid) and their characterization by various analytical techniques to evaluate their solubility properties.

Methods: Mixtures were prepared by the solvent evaporation method where the drug and the excipients are dissolved in a suitable solvent and evaporated on a low heat flame. The mixture thus obtained is vacuum dried, placed in a dessicator overnight and stored at -20°C for 4 days to ensure crystallization. Samples are then ground in a mortar and pestle and analyzed for their physiochemical properties by various analytical techniques such as DSC, TGA, SEM, XRD and UV Spectrometry.

Results: Mixtures of the drugs Nortriptyline and Quetiapine fumarate were successfully prepared. The blending of the drug with an excipient or a polymer produced a novel product with unique thermal analytical properties and solubility. Differential Scanning Calorimetry, Thermal Gravimetric Analysis (properties) as well as Wide Angle X-Ray Diffraction plus optical microscopy confirmed the structure-property relationship of the

new blend.

Conclusion: An increase in the solubility and dissolution properties were seen as a result of the reduction in particle size of the drug. Here the drug gets incorporated into the interstitial spaces of the carrier molecule and gets rapidly released. A further increase in the crystallinity which results due to solubility was also observed for some mixtures. These new blends, which were prepared, produced compounds which can be used to treat bipolar disorder as well as utilizing the therapeutic properties of the excipients used. Thus, this study proposes a new kind of formulation technique that can increase the solubility and dissolution characteristics of poorly soluble drugs while possibly introducing multiple drug therapy

29. Transoral Patch Development and Kinetic Study of Drug Release in Artificial Saliva

Arpana Acharya^[1], Kenneth S Alexander^[1], Alan T Riga^[1,2]. ^[1]Department of Pharmacy Practice, The University of Toledo, Toledo OH; ^[2]Department of Clinical Chemistry, Cleveland State University, Cleveland, OH.

Purpose: The development and characterization of buccal films loaded with lidocaine were the objectives for this study. Lidocaine has analgesic properties and is a poorly water soluble drug. If polymeric systems are selected appropriately, they can serve as a carrier for lidocaine molecules for more effective drug delivery in the buccal cavity. The key limitations to this approach are the balance in hydrophobicity and solubility parameters between the polymer and the drug and mimicking of appropriate physico-chemical properties in selecting the dissolution media.

Methods: A transparent, thermoplastic polymer, Polymethylmethacrylate, was dissolved in acetone (50% w/w) and the solution was poured into a Teflon-coated pan using an inverted funnel on top to ensure slow evaporation. Thickness and weight variation of the films, both with and without drug, were evaluated. Thermal analytical techniques such as Differential Scanning Calorimetry and Thermo-Gravimetric Analysis were used to evaluate film reproducibility and homogeneity. Scanning Electron Microscopy was used to determine the size and texture of the polymeric film. The release kinetics of the drug from the film was studied with the help of a dissolution study in artificial saliva. The experiments were extended to the Eudragit S-100

polymer as well.

Results: The thermo-analytical and microscopic techniques indicated that the drug was successfully loaded and the prepared films were homogeneous and reproducible. The data obtained from the dissolution study was used to model the kinetics of the system using multiple equations such as Higuchi, first order, zero order and korsmeyer-peppas functions. The release of drug followed a first order reaction which was concluded from statistical analysis using the goodness-of-fit approaches.

Conclusion: The approach of using a polymeric carrier in delivering lidocaine via buccal delivery was demonstrated using in-vitro techniques. Future work should evaluate the impact of plasticizer on modifying the rate of drug release from these polymer films.

30. Evaluation of Powder Porosity and Void Space in Pharmaceutical Dispersions by Volumetric Variation Method

Zhouyuan Liu⁽¹⁾, K.S. Alexander⁽³⁾ and A.T. Riga⁽²⁾. ⁽¹⁾⁽³⁾ College of Pharmacy, Pharmacy Practice Department, Industrial Pharmacy Division, University of Toledo, Toledo, OH, USA; ⁽²⁾ Department of Clinical Chemistry, Cleveland State University, OH, USA.

Purpose: The traditional method, namely sedimentation, is used to determine the porosity and void space of powder particles in pharmaceutical dispersions. This allows the medium to fill the voids within the powder particles, and also allows time for particle -particle-suspending medium association for equilibrium to be established in the system. In order to simplify the process and save time, the titration method to determine the porosity of powders was developed by T. Huyhn and K. Alexander. Utilizing a beaker and burette, the titration method directly measures the immediate pore volume between the particles rather than determining the solvent taken up in the pores and the coagulation of the particles. The titration method is time-saving, however, no study has shown that it is effective and accurate. The purpose of this study is to evaluate the validity and sensitivity of the titration method in order to determine the porosity of powders.

Methods: TGA and X-ray Diffraction were used to characterize the powders. Multiple titrations using various powders and pharmaceutical liquid mixtures were performed utilizing various sized beakers and burettes. Magnesium oxide and charcoal were used

along with various solvent mixtures. Binary mixtures were prepared using Octanol-Ligroine, Methanol-Isopropanol, and Glycerin-Water with compositions which varied from 100% of one ingredient to 100% of the other. Glass beads were used as the control group. The properties of the liquid mixtures including surface tension, viscosity and polarity, and the properties of the powders including particle size were also determined. Various statistical methods were involved to analyze the data, including linear regression and multiple linear regression.

Results: The results were plotted as weight of solid versus volume of liquid used. The data was analyzed based on the y-intercept values. The statistical analysis shows the y-intercept is zero for glass beads, but for porous powders the y-intercept was never equal to zero. Non-zero y-intercepts imply that the different solvents, as measured by polarity and dielectric constant, have varying particle-liquid attractive forces. The polarity of the solvent affects the results as well as other parameters such as viscosity of the liquid and particle size of the powders. A mathematic model was established utilizing multiple regression.

Conclusion: The results of the study indicate that the titration method is more effective for non-porous powders. For porous powders, the properties of the liquid and powder affect the determination of the porosity of the powders in pharmaceutical dispersions.

31. Novel Approach to the Characterization of Pharmaceutical Excipients Reveals New Dielectric Visco-elastic Properties by Thermal Analytical Methods

Alan Riga^[1,2], Kenneth Alexander^[1]. ^[1] Division of Industrial Pharmacy, Department of Pharmacy Practice, College of Pharmacy, University of Toledo, Toledo, Ohio USA; ^[2] Department of Chemistry, Cleveland State University, Cleveland, Ohio USA.

Purpose: Millions of dollars are expended on pharmaceutical testing to qualify excipients for fully formulated drugs, medicines, and active ingredients. Individual and interactive properties of excipients and drugs are needed to predict their action in the human body at body temperature 37°C. Dielectric Analysis [DEA] and Differential Scanning Calorimetry [DSC] methods were employed to screen the most widely used drug excipients seeking

new properties to assist pre-formulation studies.

Methods: The following excipients were examined by DEA: calcium phosphate, cotton seed oil, croscarmellose, gelatin, mannitol, peanut oil, polyethylene glycol, pioneer sugar, plasdane, sodium alginate, sodium lauryl sulfate, sodium starch glycolate, sodium stearate, a sorbitol solution, canola oil, anhydrous lactose, and benzoic acid. A comparison of DSC and DEA thermal curves based on the same excipient indicates that major endothermic events, e.g. volatilization or melting, are also delineated by fundamental DEA properties, with an exponential rise in permittivity and dielectric loss factor (conductivity).

Results: The focus of this research and development was to study the “thermal event” response from the DEA, that is, the electrical conductivity, permittivity and tan delta vs. frequency as a function of temperature. The premelt DEA properties varied significantly and repeatably leading to interesting new dielectric visco-elastic properties.

Conclusion: Crystalline excipients have a low electrical conductivity (ca. 10^{-1} pS/cm) while their amorphous form has an exceptionally high electrical conductivity (ca. 10^7 pS/cm). Relative amorphous and crystalline content of the excipients can be determined by DEA. We discovered that there is an extraordinary event in the solid state prior to melting which can be associated with the creation of excited molecules (an excimer like formation). This revelation can lead to new synthesis and reaction paths.

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32. Effect of Obesity on the Pharmacological Response to Cardiovascular Drugs in Pediatric Patients with Renal Disease

Sherif Hanafy¹, Maury Pinsk², Fakhreddin Jamali¹. ¹ Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada; ² Department of Pediatric Nephrology, Stollery Children's Hospital, University of Alberta, Edmonton, Alberta, Canada.

Purpose: Obesity has been found to be associated with increased concentration of inflammatory mediators. In adults, increased in these mediators is

shown to reduce response to calcium channel blockers. We studied the effect of obesity on the pharmacodynamic response to L-type calcium channel blockers (CCBs), angiotensin interruption agents (ANGI, angiotensin converting enzyme inhibitors or angiotensin II receptor blockers) or combination of the two in pediatric patients.

Methods: We undertook a retrospective chart review of 263 nephrology patients at the Pediatric Nephrology Outpatient Clinic at University of Alberta Hospital/Stollery Children's Hospital ; 48 patients (25 obese and 23 non-obese) were included. The lowering blood pressure effect of the antihypertensive treatment on obese were compared with non-obese patients.

Results: The systolic response to CCBs, measured as at least 10% reduction from the baseline, was significantly less in obese (12.5%) as compared with the non-obese (52.9%) group. The diastolic response, although numerically different (58.8 and 25% for non-obese and obese, respectively) did not reach significance. The percent response to CCBs, however, was significantly less in the obese as compared with the non-obese patients for both systolic and diastolic blood pressure. Corticosteroids also had significant influence on diastolic response to CCBs (62.9 and 25% for non-obese and obese, respectively). None of the tested covariates, including obesity, was found to significantly influence the response to ANGI alone or in combinations with CCBs.

Conclusion: Obesity and corticosteroid therapy are important confounding factors that govern the responsiveness of pediatric patients with renal disease to hypotensive drug therapy. Obesity should be considered when initiating drug therapy for children with kidney disease.

33. Differences in Prevalence and Treatment Patterns of Lower Extremity Peripheral Arterial Disease in Urban and Rural Communities in Alberta (epiPAD)

Trevor Elton, BSc, MSc¹; Mark Makowsky, BSP, PharmD^{1,2}; Sean McMurtry, MD, PhD²; Ross Tsuyuki, BSc(Pharm), PharmD, MSc.^{1,2} ¹Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada; ²EPICORE Centre/COMPRIS, Division of Cardiology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada.

Purpose: Peripheral arterial disease (PAD) is a

marker of atherosclerotic vascular disease and is associated with increased risk of cardiovascular events. American and European data suggests several gaps in the care of individuals with PAD, particularly under-diagnosis and under-treatment. People living in rural settings generally have poorer health, poorer health outcomes and have less access to healthcare services. We sought to determine if there are differences in the prevalence and treatment patterns of PAD in urban and rural community settings in Alberta.

Methods: We screened subjects over 50 years of age in five urban and four rural pharmacies in Central and Northern Alberta during July and August 2008. We gathered demographic information, assessed cardiovascular risk factors, collected current medications, administered the Edinburgh Claudication Questionnaire, and measured the ankle brachial index using a Doppler ultrasound (Model L150, Summit Doppler Inc). PAD was defined as an ABI <0.90. Our primary outcome was to compare the prevalence of PAD between individuals living in urban and rural areas, while secondary outcomes were to compare use of evidence-based therapies (ASA, statins, ACE inhibitors) between these two groups.

Results: We recruited 203 patients (98 rural, 105 urban). The mean age was 66.2 ± 9.3 years and 37.9% were male. Overall, patients were similar in the two groups with 125 (61%) being current or former smokers, 44 (22%) having diabetes, 97 (48%) dyslipidemia, and 117 (57%) hypertension. We identified 5 urban patients and 5 rural patients with PAD (prevalence 4.8% vs. 5.1%; $p = 0.911$). All 10 patients (100%) were previously undiagnosed and 7 (70%) had claudication symptoms. Due to the small number of patients with previous PAD, our investigation of treatment patterns was limited in these patients. In all patients with PAD, 7 (70%) were using ASA, 5 (50%) an ACE inhibitor, and 6 (60%) a statin. Only 3 (30%) were receiving all three evidence-based therapies.

Conclusions: There were fewer cases of PAD in the community setting than initially anticipated, and we found no evidence of differences in prevalence between urban and rural settings. However, PAD is rarely assessed in the community and pharmacists could play a role in screening and improving application of medications with proven mortality benefit for this important condition.

34. An Interim Analysis: The Role of Superantigen Producing *Staphylococcus aureus* in the Etiology of Multiple Sclerosis

Michael Prout², Christine Leong², Meghann Klowak², Amy Grossberndt², Maria Melanson¹, Andrew Gomori¹, Farid Esfahani¹, Loressa Klassen¹, Yuewen Gong², Malcolm Doupe³, Romeo Hizon⁴, Michael Mulvey⁴, Michael Namaka^{1,2}. 1 Health Sciences Centre, Department of Neurology, Winnipeg, Manitoba; 2 University of Manitoba, Faculty of Pharmacy, Winnipeg, Manitoba; 3 Manitoba Centre for Health Policy, University of Manitoba, Winnipeg, Manitoba; 4 National Microbiology Laboratory, Antimicrobial Resistance and Nosocomial Infections, Winnipeg, Manitoba.

Background: Multiple sclerosis is an autoimmune disease characterized by the T-cell targeted destruction of central nervous system (CNS) myelin. Epidemiologic studies have led some investigators to suggest that environmental factors, including infectious diseases, may be associated with the underlying etiology of MS. This theory has developed from the correlation between geographical location and the prevalence of MS. As a result, it is plausible that patients residing in specific geographical locations may be predisposed to exposure of organisms such as superantigen producing *Staphylococcus aureus* (*S. aureus*). This is supported by in vitro studies, which have demonstrated that *S. aureus* superantigens stimulate the same subset of T cells responsible for the demyelination associated with MS. In the absence of infection, toxin or superantigen secreted by nasal colonizations of *S. aureus* may invade the systemic circulation and through the process of molecular mimicry, they may trigger an autoimmune response against CNS myelin.

Purpose: The primary objective of this study is to determine if nasal carriage rates for *S. aureus* correlate with acute exacerbations of MS. The specific objective of this research is to determine whether there is a similarity between the various types of *S. aureus* producing toxins found in patients undergoing an acute exacerbation of MS.

Methods: A comparative, single centre, open-label study was conducted on participants diagnosed with relapsing-remitting MS (RRMS) to assess nasal colonization rates of *S. aureus*. According to the study design, 240 participants were scheduled for recruitment. Study participants were divided into three groups: *naïve control* (n=80), *active control*

(n=80) and an *acute exacerbation group* (n=80). Nasal colonization rates were assessed by swabbing the bilateral nares of participants from each of the three groups. Polymerase chain reactions (PCR) were used on these samples to determine the toxin genotype for all *S. aureus* isolates. Finally, pulsed-field gel electrophoresis (PFGE) was conducted following the standard method on all isolates to determine the molecular relatedness of strains. Data was stored and compared using BioNumerics software (Applied-Maths). A multi-variate logistic regression was used to compare the odds that participants tested positive for *S. aureus* in each study group, while controlling for the previously cited differences in patient demographics (age, sex, ethnicity), disease and drug use. The primary study outcome is the difference in nasal colonization rates and associated toxins between the naïve control group, the active control group and the acute exacerbation group.

Results: An interim analysis of the results (naïve control n = 80, active control n=80 and acute exacerbation group n = 43) suggests an increase in nasal carriage rates for *S. aureus* in MS patients compared to controls. These rates appear to further increase during an MS attack.

Conclusion: The ability to establish correlations with specific superantigen producing strains of *S. aureus* identifies a novel mechanism by which antimicrobial treatment strategies can be used to attenuate acute exacerbations of MS. The results of this research may have significant potential in improving patient outcomes by slowing progression of a disease that is inherently prevalent within their geographical location.

Pharmaceutical and Analytical Chemistry

35. Structural Elucidation of Drug Metabolites and Identifying New Antibiotics Using Mass Spectrometry

Thomas Veinot¹, Stephanie Dupuis² and David Jakeman^{1,2}, Pauwlin Dexter¹, Marie-Paule Gilles¹ and Hoan Linh Banh¹. ¹College of Pharmacy, Dalhousie University; ²Department of Chemistry, Dalhousie University, Dalhousie, NS, Canada.

Purpose: To help establish the structures of

propofol metabolites in patients presenting with green urine, as well as to identify new antibiotics from bacterial cultures using MS / MS mass spectrometry (MS).

Methods: Samples of either the propofol metabolite or the antibiotic were prepared for mass spectrometry analysis. The mass spectrometer was used to perform a series of scans designed to determine the major components in each sample. The signals corresponding to each component were then subject to further MS/MS analysis to determine their breakdown patterns and more accurately identify the structures of the molecules present.

Results: Based on the graphs generated by the propofol samples there were four major peaks of interest at 167, 209, 335 and 532 m/z amu. While there was no clear breakdown of the two lower masses, the two higher amounts corresponded to fragments of known Propofol metabolites. Analysis of spectra from the bacterial cultures identified the predicted signals for new antibiotics incorporating D-norleucine and D-norvaline amino acids, however samples isolated from bacterial cultures grown on L-norleucine and L-norvaline did not contain the anticipated antibiotics.

Conclusions: The urine containing propofol metabolites was determined to have several known metabolites including propofol glucuronide. In addition, there was spectral evidence to indicate that there may be new, previously unobserved, metabolites, however, further HPLC-MS analysis will be required to unequivocally confirm these observations. We determined that cultures of the bacterium *Streptomyces venezuelae* ISP5230 were able to metabolize D-norleucine and D-norvaline into antibiotics, but not L-norleucine or L-norvaline.

Acknowledgements: We thank the Pharmacy Endowment Fund

Disclosure: The authors have nothing to disclose concerning financial or personal relationships with commercial entities:

36. Thermodynamic Studies of Nitroxoline Sublimation by Simultaneous DSC-FTIR Method and Isothermal TG Analysis

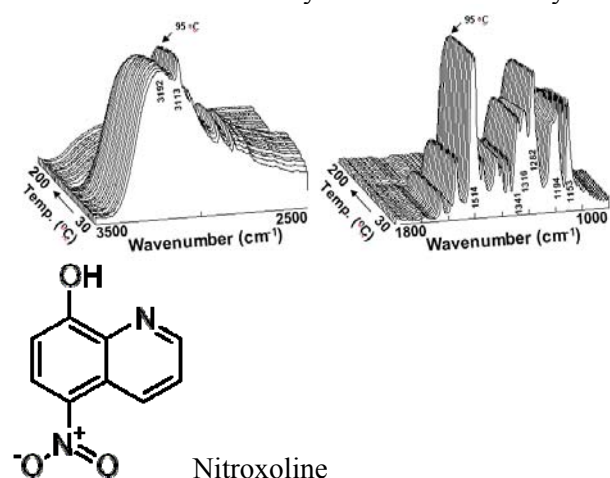
Gau-Yi Gao and Shan-Yang Lin*. Lab. Pharm. Biopharm., Department of Biotechnology, Yuanpei University, Hsin Chu, Taiwan, Republic of China.

Purpose: To investigate the physicochemical characteristics, thermodynamics, possible sublimation process and kinetics of nitroxoline.

Methods: Differential scanning calorimetry (DSC), isothermal thermogravimetry (TG), and Fourier transform infrared (FTIR) microspectroscopy equipped with a micro hot-stage of DSC microscopy assembly (simultaneous DSC-FTIR method) were used.

Results: The DSC result indicates that nitroxoline exhibited a sharp endothermic peak at 182°C with enthalpy of 103.1 J/g due to the melting point of nitroxoline. A sublimation behavior of nitroxoline was found from 129° by gradual weight loss in TG curve. However, the non-isothermal DSC-FTIR method reveals that the temperature at 95° was the onset temperature of nitroxoline sublimation. A significant difference between DSC-FTIR method and TG analysis suggests that the simultaneous DSC-FTIR method. The sublimation kinetics of nitroxoline determined by isothermal TG analysis evidenced that the zero-order kinetics was followed over the sublimation time. The enthalpy of nitroxoline sublimation was 81.66 KJ/mole, but its entropy was 177.8 J/mole.

Conclusion: The simultaneous DSC-FTIR method was easily used to investigate the sublimation process of nitroxoline and also exhibited a higher sensitive technique than that of the TG analysis to detect the beginning temperature of nitroxoline sublimation. The Eyring equation was available applied to estimate the sublimation kinetics of nitroxoline determined by isothermal TG study.



Three-dimensional plots of FTIR spectra of nitroxoline in the range of 3500-2500 and 1800-1000 cm⁻¹ as a function of temperature.

Acknowledgements: This work was supported by National Science Council, Taipei, Taiwan, Republic of China (NSC-95-2320-B075-002-MY2).

37. Similarities and Differences in the Mass Spectrometric Fragmentation Patterns of a Series of Novel Gemini Surfactants Used in Non-Viral Gene Delivery

Joshua Buse¹, Ildiko Badea¹, Ronald R. Verrall², Anas El-Aneed¹. ¹College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK;. ²Department of Chemistry, University of Saskatchewan, Saskatoon, SK.

Purpose: The establishment of the tandem mass spectrometric (MS/MS) fingerprints of thirty novel gemini surfactant nanoparticles, that were selected from ten unique structural families. *Hypothesis:* Each structural family of gemini surfactants will produce unique fragmentation patterns during MS/MS analysis and each gemini surfactant will produce distinctive product ions.

Methods: Each gemini surfactant was dissolved in 50:50 methanol and Millipore water with 0.1% fluoric acid to a concentration between 2-3 μM and stored at 4° C prior to analysis. Samples were injected into an Applied Biosystems API QSTAR XL MS/MS, set in the positive ion mode, at an infusion rate of 10 $\mu\text{L}/\text{minute}$. The declustering potential was 40.0 and the focusing potential was 290.0. The source voltage was 5.5 kV with a temperature between 80° C and 100° C. Argon was used as a collision gas and the collision energy was varied between 15 and 100 eV; in order to ensure the observation of both the molecular ion and product ions at abundant levels. Data analysis was performed utilizing the Analyst® software.

Results: The gemini surfactants within a unique structural families followed a universal fragmentation pattern and each gemini surfactant produced unique product ions that allowed for their individual identification. Common elimination products which were continuously observed included product ions that resulted from the loss of the gemini surfactant's tail region, loss of both its tail regions, and fragmentation within its spacer region. In addition, as the mass of the gemini surfactants increased, a general expansion in the number of product ions, which indicated the presence of additional fragmentation pathways, was observed. The unique product ions produced through the MS/MS analysis of each gemini surfactant allowed for their structural identification.

Conclusion: The MS/MS analysis of each gemini surfactant produced a unique MS/MS fingerprint which followed a universal fragmentation pattern for

each structural family of gemini surfactants. These MS/MS fingerprints will assist in the development of liquid chromatography MS/MS methods and the quantification of each gemini surfactant within biological systems. In addition, the collected data will facilitate metabolite bi-product(s) identification.

38. A Validated UV-Spectrophotometric Method for the Simultaneous Quantification of Flurbiprofen and Sparfloxacin in Bulk Drug

Jasjeet Kaur Sahni, Mohammad Tariq, Zeenat Iqbal and Farhan.J.Ahmad. Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi, India.

Purpose: Sparfloxacin, is a broad spectrum antibiotic and flurbiprofen, is a significant inhibitor of inflammation and alveolar bone loss. The two drugs have therefore been formulated as combination therapy and a periodontal drug delivery device containing both the drugs is being developed in our laboratory. Literature review reveals that no formulation containing these two drugs in combination has been reported and therefore no method of simultaneous estimation of flurbiprofen and sparfloxacin has been reported. The purpose of this research was to establish and validate a simple, accurate, economical, and reproducible spectroscopic procedure for simultaneous estimation of flurbiprofen and sparfloxacin in bulk drug, in accordance with ICH guidelines.

Methods: The Q value analysis method was used for development of UV-spectroscopic method for the simultaneous quantification of flurbiprofen and sparfloxacin in bulk drug. From the overlain spectra of drugs, two wavelengths were selected, one at 247.5 nm (the λ_{max} of flurbiprofen) and the other at 265 nm (the iso-absorptive point for both the drugs). The accuracy and reliability of the method was assessed by estimating the various parameters as per ICH guidelines.

Results: Beer's law was obeyed in the concentration ranges of 1–20 $\mu\text{g}/\text{ml}$ for both drugs and correlation coefficients were found to be 0.998 and 0.995 for flurbiprofen and sparfloxacin respectively. The apparent molar absorptivity and absorptivity coefficient were found to be $2.09 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $3.40 \times 10^2 \text{ gm}/\text{dl}$ for flurbiprofen and $1.33 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $3.40 \times 10^2 \text{ gm}/\text{dl}$ for sparfloxacin in phosphate buffer pH 7.4. The limit of detection and limit of quantification of flurbiprofen and sparfloxacin were found to be 0.2887 $\mu\text{g}/\text{ml}$ and

0.866 µg/ml and 0.2937 µg/ml and 0.881 µg/ml, respectively. The accuracy of method was assessed by recovery studies by taking the mean of 6 replicates of mixtures containing the combination of the two drugs in different concentration ratios and was found in the range of 99.5-102.7 % with SD ± 1.412 and 99.8-102.5% with SD± 1.017 for flurbiprofen and sparfloxacin respectively. The accuracy of method was also assessed by recovery studies at different days by taking the mean of 6 replicates of the above mixtures and was found to be 100.35% with mean SD ± 0.877 & RSD ± 0.873 and 100.94% mean SD ± 0.79 & RSD± 0.782 of flurbiprofen and sparfloxacin respectively.

Conclusion: The results demonstrated that the developed method for the simultaneous quantification of flurbiprofen and sparfloxacin in bulk drug is simple, rapid, accurate, precise, specific and reproducible (%RSD <2%).

39. High Performance Liquid Chromatographic Method Development and Validation of Cimetidine in Human Plasma for Bioequivalence Study

¹*Tanveer Ahmad Khan ²Abul Bashar Mohammad Helal Uddin, and ³Syed Atif Abbas. ¹College of Clinical Pharmacy, King Faisal University, 31982 Al-Ahsa, Kingdom of Saudi Arabia; ²Department of Pharmaceutical Chemistry, Kulliyah of Pharmacy, International Islamic University, Pahang, Malaysia;

³School of Biomedical Sciences, Charles Sturt University, Orange New South Wales, 2800 Australia.

Purpose: The aim of this study was to develop a validated and simple method for the determination of cimetidine in human plasma for bioequivalence study.

Methods: A reverse phase high performance liquid chromatography (RP- HPLC) method for the detection of cimetidine was performed from human plasma spiked with standard cimetidine. Ranitidine was used as an internal standard. Simple liquid-liquid extraction method was adopted for the isolation of the cimetidine from the human plasma. The extraction method was compared with direct precipitation method using mineral acids and organic solvents. Chromatographic separation was carried out by a C₁₈ reversed-phase column; UV detection was set at 228 nm. The mobile phase was composed of sodium di-hydrogen phosphate buffer together with 5% acetonitrile. 1% tri-ethylamine was

used to avoid tailing effect and pH was optimised at 3.5. Specificity of the cimetidine and internal standard was established by comparing the standard peaks with blank plasma chromatogram. The working range of cimetidine was between 40 to 4000ng/ml in human blood. Method validation was performed for *intra*- and *inter*- day precision of the analyte, and coefficient of variance (CV) were lower than 10% for low (80 ng/ml), medium (2000 ng/ml) and high (3600 ng/ml) concentrations.

Results: The method of extraction was simple and less hazardous as compared to direct precipitation method. Liquid-liquid extraction process was found to produce a cleaner sample with lower background noise. All calibration curves showed good linear regression ($r^2 > 0.990$) within test ranges. The limit of quantitation (LOQ) concentration was 40 ng/ml, which was same as the lower point of the calibration concentration. CV for LOQ was below 15%. The recovery for both the analytes were satisfactory and suitable for bioanalytical study.

Conclusion: This simple and reproducible analytical method can be utilized for the bioequivalence studies of cimetidine in human plasma.

40. Validation of a Liquid Chromatography Tandem Mass Spectrometry Method for the Determination of Atovaquone in Human Plasma

Sandrine A. Mérette*, Grace van der Gugten, Hong Zhang, Winnie Liu, Rong Yi, Gina de Boer. CANTEST, Burnaby, BC, Canada.

Purpose: To develop and validate an LC-MS/MS method for the quantitative determination of atovaquone in human plasma. Atovaquone is a hydroxy-1,4-naphthoquinone, an analog of ubiquinone, with antipneumocystic activity. Atovaquone is also used for the treatment of toxoplasmosis or malaria, when given in combination with proguanil under the trade name of Malarone®.

Methods: Atovaquone and d₅-Atovaquone (internal standard) were extracted from 50 µL of human plasma using protein precipitation. The analyte was chromatographically separated on a Zorbax Eclipse C₁₈ (4.6 x 50 mm) column using gradient elution with a mobile phase composed of 0.1% formic acid in water and 0.1% formic acid in acetonitrile, at a flowrate of 1.5 mL/min for a total runtime of 3 minutes. LC-MS/MS was performed using Electrospray ionization (ESI) in the negative mode.

Detection and quantitation were carried out by multiple reaction monitoring (MRM) scan at 365.19 to 337.00 (Atovaquone) and 370.08 to 342.00 (d_5 -Atovaquone).

Results: The method was validated over the range of 2 to 2000 ng/mL. Inter-batch accuracy (%RE) and precision (%CV) for standards ranged from -3.0 to 3.8 and 2.6 to 6.3, respectively and quality control samples ranged from 3.3 to 6.1 and 1.8 to 2.7, respectively. The mean (n=5) correlation coefficient

was 0.9989 ± 0.0005 . Matrix effects were negligible with a mean value of -3.9%. Mean assay recovery was at $101.6 \pm 5.6\%$ (n = 15) for the analyte and $100.0 \pm 3.9\%$ (n=10) for the IS. Atovaquone stability in solution and in matrix was established.

Conclusion: Validation of a Liquid Chromatography Tandem Mass Spectrometry Method for the Determination of Atovaquone in Human Plasma was completed successfully.

Poster Presentations
Day 2
Friday, June 5, 2009

Day 2

Biomedical Sciences

41. The p38 MAPK Inhibitor SB203580 is a Novel Ligand of the Aryl Hydrocarbon Receptor

Hesham M. Korashy¹, Anwar Anwar-Mohamed² and Ayman O.S. El-Kadi². ¹Department of Pharmacology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia; ²Faculty of Pharmacy & Pharmaceutical sciences, University of Alberta, Edmonton, Alberta, Canada.

Cytochrome P450 1A1 (Cyp1a1) is a xenobiotic metabolizing enzyme which plays a major role in bio-activating pro-carcinogens and environmental pollutants into carcinogenic intermediates through activation of a ligand-dependent transcription factor, aryl hydrocarbon receptor (AhR). Recently, we have reported the first observations that SB203580, a pharmacological inhibitor of the p38 mitogen-activated protein kinase (MAPK), induced Cyp1a1 mRNA in murine hepatoma (Hepa 1c1c7) cells (Korashy HM and Al-Kadi AO. The role of redox-sensitive transcription factors NF- κ B and AP-1 in the modulation of the *Cyp1a1* gene by mercury, lead, and copper. Free Radic Biol Med. 2008; 44:795-806). Yet, the mechanisms involved remain unknown.

Purpose: To explore the molecular mechanisms involved in the modulation of *Cyp1a1* by SB203580.

Methods: Hepa1c1c7 cells were treated with increasing concentrations of SB203580 (0, 1, 5, 10, and 20 μ M) or TCDD (1 nM), a potent AhR ligand. The Cyp1a1 mRNA and protein levels were determined by RT-PCR and Western blot analysis, respectively, whereas the catalytic activity was measured in intact living cells using 7-ethoxyresorufin as a substrate. Transcriptional regulation of cyp1a1 mRNA was examined using a transcription inhibitor, actinomycin D (5 μ M). The direct activation of AhR by SB203580 was assessed using luciferase activity and electrophoretic mobility shift assay (EMSA).

Results: SB203580 significantly induced *Cyp1a1* gene expression at the mRNA, protein and catalytic

activity levels in a concentration-dependent manner. The increase in Cyp1a1 mRNA by SB203580 was completely abolished by the transcription inhibitor, actinomycin D, implying an increase in the *de novo* RNA synthesis. Furthermore, our results showed that SB203580-mediated induction of Cyp1a1 is an AhR-dependent, in which SB203580 induced the activation of the AhR-driven luciferase activity which was further reflected by the ability of SB203580 to increase the cytosolic transformation of AhR and subsequent activation of the xenobiotic responsive elements in *Cyp1a1* promoter.

Conclusion: SB203580 induced the expression of *Cyp1a1* gene at the transcriptional level through an AhR-dependnet mechanism and hence considered as a novel AhR ligand.

Acknowledgments: CIHR/Rx&D, NSERC, University of Alberta, and King Saud University

42. An *in vivo* Evaluation of an APN/CD13-Targeted CT Contrast Agent

Michael Dunne¹, Jinzi Zheng², David Jaffray^{2,3}, Christine Allen^{1,4}. ¹Department of Pharmaceutical Sciences, University of Toronto, Toronto, ON; ²Department of Medical Biophysics, University of Toronto, Toronto, ON; ³Department of Radiation Physics, Princess Margaret Hospital, Toronto, ON; ⁴Department of Chemistry, University of Toronto, Toronto, ON.

Purpose: Angiogenesis is an essential process in malignant tumour development. This work attempts to develop a contrast agent capable of monitoring this process. Aminopeptidase N (APN) is a transmembrane metalloprotease overexpressed on endothelial cells lining neovasculature. The Asp-Gly-Arg (NGR) peptide motif has been shown to selectively bind to the APN isoform expressed on tumour neovasculature. This study compares four liposome formulations that accumulate at the tumour site due to the enhanced permeation and retention effect as well as by active targeting using NGR. Studies using these contrast agents can improve the design of NGR-therapeutics currently in clinical and preclinical development by tracking the agent throughout the body at many times over a prolonged period.

Methods: Four liposome formulations were prepared by the extrusion method and characterized *in vitro* in terms of size, size distribution, zeta

potential, and stability. These formulations were composed of 55:40 mol% DPPC:cholesterol with a further 5 mol% composed of both PEG₂₀₀₀-DSPE and the following targeted lipids (1) no targeted lipid (2) 0.64 mol% NGR-PEG₂₀₀₀-DSPE (3) 2.56 mol% NGR-PEG₂₀₀₀-DSPE (4) 0.64 mol% NGR-PEG₃₄₀₀-DSPE. Iohexol, an iodinated CT contrast agent approved for clinical use, was encapsulated inside the liposomes. These formulations were administered intravenously to athymic CD-1 mice bearing subcutaneous H520 human non-small cell lung cancer tumours on their right hind legs. CT imaging was performed ten times during one week. Contrast agent accumulation at the tumour site was calculated by comparing contrast enhancement in the tumour volume to that of standards of known concentration. Similar methods were used to determine contrast agent concentrations in the blood pool and healthy organs.

Results: All formulations were approximately 90 nm in diameter with a unimodal distribution. Maximum tumour accumulation of formulations 1-3 occurred 48 hr post-injection. The tumour accumulation kinetics of formulation 4 were altered such that contrast agent concentration continued to increase up to 72 hr. The highest iodine concentration observed in the tumour was formulation 2 at 48 hr.

Conclusion: *In vitro* work has confirmed that there is little difference between the formulations in terms of size and stability, yet all formulations perform differently post-injection. This work demonstrates there is an optimal density of targeting ligands on the vehicle surface such that increasing or decreasing will result in less tumour accumulation. It also suggests that tumour residence time can be altered by including a spacer between the targeting ligand and the PEG sheath surrounding the liposome.

43. Inhibition of the OATP2B1 Influx Transporter by HIV-1 Protease Inhibitors in Caco-2 Cells

O. Kis¹, J. A. Zastre², A. Otting¹, M. Ramaswamy¹, R. Bendayan¹. ¹Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto; ²Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia.

Purpose: Currently, limited information is available on the mechanism of intestinal uptake of HIV-1

protease inhibitors (PIs) and their interactions with influx transporters. Several members of the organic anion transporting polypeptide (OATP) family are expressed in enterocytes, with OATP2B1 expressed at the highest level. We examined the ability of PIs to act as substrates and/or inhibitors of OATP2B1 system in Caco-2 cells, an established *in vitro* model of intestinal epithelium.

Methods: Expression of OATP2B1 in Caco-2 and OATP2B1-overexpressing MDCKII cells was confirmed by RT-PCR and immunoblot analysis. The function of OATP2B1 was assessed through uptake studies using radiolabeled estrone-3-sulfate (E3S), an established probe for this system. Applying a similar approach, uptake of 1 μ M [³H]atazanavir or ritonavir was evaluated. Antiretroviral drugs were screened for interactions with OATP2B1 by measuring [³H]E3S uptake in the presence or absence of each drug. IC₅₀ concentrations were determined from concentration-dependent inhibition studies. The effect of proton gradient on OATP2B1-mediated transport of E3S was assessed by varying extracellular pH; dissipating the pH gradient with the Na⁺/H⁺ ionophore, monensin (5 μ M), and acidifying the intracellular fluid by NH₄Cl preincubation (30 mM).

Results: OATP2B1-mediated transport of estrone-3-sulfate in MDCKII/OATP2B1 and Caco-2 cells had comparable transport characteristics ($K_M \approx 13 \mu$ M). An inwardly directed H⁺ gradient was identified as the driving force for the uptake. E3S transport was potently inhibited by PIs, lopinavir, tipranavir, and nelfinavir, with IC₅₀ concentrations below 2 μ M, and atazanavir and ritonavir, with IC₅₀ values of 2.2 μ M and 3.9 μ M, respectively. While atazanavir accumulation in Caco-2 cells was significantly enhanced at lower extracellular pH, its uptake by MDCKII/OATP2B1 cells was not significantly different from the wild type, suggesting that OATP2B1 is not involved in the intestinal uptake of atazanavir.

Conclusion: Although the intestinal uptake of atazanavir and ritonavir is not mediated by OATP2B1, these PIs, as well as lopinavir, tipranavir, and nelfinavir, are potent inhibitors of this transporter. Since OATP2B1 exhibits an increasing number of drug substrates, including statins, inhibition of its function by PIs could result in clinically important drug-drug interactions in the intestine.

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44. Ethanol Embryopathies, Oxidative Stress and Protection by a Free Radical Spin Trapping Agent

Lutfiya Miller¹ and Peter. G. Wells^{1,2}. ¹Department of Pharmacology and Toxicology and ²Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada.

Consumption of alcohol (ethanol) during pregnancy can cause a spectrum of structural, cognitive and behavioural anomalies termed the Fetal Alcohol Spectrum Disorder (FASD). Although the underlying mechanisms are not fully understood, there is evidence suggesting that reactive oxygen species (ROS) are involved in the teratogenic mechanism.

Purpose: To determine the extent to which ROS are involved in FASD.

Methods: CD-1 mouse embryos were explanted on gestational day (GD) 8 (plug = GD 0) and exposed over 24 hr to a range of ethanol concentrations (0, 2, 4, 6 mg/ml) or its vehicle, with or without preincubation with phenylbutylnitron (PBN), a free radical spin trapping agent previously shown to reduce embryonic ROS formation and embryopathies caused by teratogens like phenytoin and thalidomide.

Results: Ethanol caused concentration-dependent embryopathies that were reduced by preincubation with PBN. Exposure to 2, 4 and 6 mg/ml concentrations of ethanol alone decreased somite development (16%, 29%, 100% reductions, respectively, $p < 0.05$) and dorsal-caudal flexion (turning) (43%, 25% and 100% reductions, respectively, $p < 0.05$). Preincubation of embryos with PBN (1.085 $\mu\text{Mol/ml}$) followed by ethanol (4 mg/ml) was protective, evidenced by increases in somite development (PBN treated=20.7, ethanol=15.7, $p < 0.05$) and turning (PBN treated=100%, ethanol=75%) compared to embryos exposed to ethanol alone.

Conclusions: The reduction in ethanol embryopathies by PBN suggests that the FASD is caused in part by ethanol-initiated embryonic ROS formation. (Support: CIHR. Abstract reproduced from Birth Defects Res. Part A [in press], 2009.)

45. Accelerated Cytotoxic Mechanism Screening (ACMS) for Idiosyncratic Hepatotoxic Drugs

Peter J. O'Brien and Shahrzad Tafazoli. Faculty of Pharmacy, University of Toronto, Toronto, ON

Purpose: Hepatotoxicity is the commonest cause of drug failure and hepatocytes are generally thought to be the gold standard for in vitro drug metabolism studies or toxicity testing. Hepatocytes freshly isolated from rats have similar phase 1 & 2 drug metabolizing enzyme activities and GSH levels as that found in vivo. It has been shown that liver inflammation activates Kupffer cells and causes neutrophil infiltration. These cells then release reactive oxygen species (ROS), myeloperoxidase and cytokines that contribute to the increase in hepatocyte susceptibility to drugs or xenobiotics during inflammation.

Methods: ACMS techniques use cell death as an endpoint to determine which metabolic pathways and defense systems affect drug toxicity. Useful rescue agents include traps for endogenous toxins e.g. carbonyls or drug reactive metabolites (O'Brien 2005 Current Drug Metabolism 6,101-111). Redox agents can also be used to normalise the cellular redox potential modulations by the toxin. Cellular redox potential can be determined from the ratios for GSH/GSSG, lactate/ pyruvate and β -hydroxybutyrate /acetoacetate. Oxidative stress can be measured by ROS formation, Fox assays and protein carbonylation. Hepatocyte bioenergetic stress can also be determined by mitochondrial membrane potential inhibition and decreased ATP/AMP ratios.

Results: A marked increase in isoniazid induced hepatocyte cytotoxicity was found when hepatocytes were preincubated with isoniazid and then exposed to a non- cytotoxic H_2O_2 generating system (glucose/glucose oxidase) so as to mimic ROS released by activated Kupffer cells. ACMS techniques showed that the cytotoxic mechanism involved mitochondrial oxidative stress and the release of lysosomal Fe and cathepsins. Cytotoxicity was prevented by BNPP, a carboxyesterase inhibitor, thereby showing that hydrazine was a toxic isoniazid metabolite. A non cytotoxic MPO/ H_2O_2 generating system markedly increased the hepatocyte cytotoxicity of troglitazone, amodiaquine, hydralazine. ACMS techniques showed that the cytotoxic mechanism involved oxidative stress that could be attributed to phenoxyl, semiquinoneimine, hydralazyl prooxidant

radicals respectively. Hepatocyte cytotoxicities induced by other drugs e.g. ibuprofen, aspirin were not affected by H₂O₂ with or without MPO.

Conclusions: This H₂O₂ hepatocyte inflammation model should prove to be a more robust screen of drug candidates for idiosyncratic hepatotoxicity potential.

46. The Effect of CYP1A Induction on Amiodarone Disposition in the Rat

Marwa E. Elsherbiny and Dion R. Brocks. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta.

Purpose: In the treatment of cardiac arrhythmias, amiodarone (AM) has emerged as a primary therapeutic agent. In addition to other cytochrome P450 (CYP), 1A1 and 1A2 facilitate the biotransformation of AM to the pharmacologically and toxicologically active metabolite, desethylamiodarone (DEA). In turn, the exposure to polycyclic aromatic hydrocarbons can induce these isoforms. This study was aimed at investigating the effect of CYP1A induction on the disposition of AM, after single and multiple intravenous doses, using β -naphthoflavone (BNF) treated rats to model the CYP1A induction.

Methods: In the single dose study, the rats were allocated into two groups. The control group, received 2 mL/kg/d of corn oil (CO) and the BNF-treated group, received 80 mg/kg/d of BNF/CO through intraperitoneal injection, for 4 days. On the fifth day, the rats were injected with a single dose (25 mg/kg) of AM HCl through jugular vein implanted cannula and serial blood samples were collected at different time points for determination of AM pharmacokinetics parameters. In the multiple dose study, the rats were allocated into two groups which received either CO alone or BNF/CO for 4 days, at the same dose level that was mentioned previously. On the third day of dosing, the rats received 25 mg/kg of AM HCl through jugular vein implanted cannula. AM dosing continued for 4 days. After the last dose blood, intestine, liver, kidneys, heart, and lung tissues were collected at preset time points and assayed for AM and DEA concentrations.

Results: After a single dose (25 mg/kg), the plasma AUC_{0-24h} and AUC_{0-∞} of DEA, were significantly increased (2.9-fold). With multiple doses, AM AUC_{0-24h} was significantly reduced in the BNF treated plasma (30%), lung (35%), liver (48%), kidney (52%), heart (34%), and intestine (43%). In

contrast, the DEA AUC_{0-24h} was increased significantly in the BNF treated plasma (36%), lung (56%), liver (101%), kidney (65%), and heart (73%). The AUC_{0-24h} of the DEA/AM was significantly increased in the BNF-treated plasma, lung, liver, kidney, and heart by 2.2, 2.8, 4.5, 4.6, and 4.2-fold, respectively. In both groups of rats, the highest concentrations of AM and DEA were in the lung.

Conclusion: Exposure to BNF was shown to increase DEA concentrations in the rat.

47. Discovery of a Potential Ribosome-Inactivating Protein Antidote

Andrew J. McCluskey^{1,2} and Jean Gariépy^{1,2,3}.¹ Department of Pharmaceutical Sciences, University of Toronto, Ontario, Canada; ² Ontario Cancer Institute, University Health Network, Toronto, Ontario, Canada; ³ Department of Medical Biophysics, University of Toronto, Ontario, Canada.

Purpose: Shiga-like toxin 1 (SLT-1) is a type II ribosome-inactivating protein (RIP), responsible for water and food poisoning, where its A₁ domain blocks protein synthesis in eukaryotic cells. The molecular mechanism leading to this event, a single depurination in the 28S rRNA, is thought to involve interactions with eukaryotic ribosomal proteins in close proximity.

Methods: In order to elucidate ribosomal protein binding partners, a pull-down using a HeLa cell lysate followed by tandem mass spectrometry was performed. The interactions and interaction sites were confirmed by yeast-2-hybrid and pull-down experiments using candidate full length and truncated proteins. This led to the discovery of a conserved C-terminal peptide binding site. *In vitro* assays for protein synthesis and cytotoxicity using the C-terminal peptide were performed to evaluate the peptide as a potential therapeutic.

Results: The mass spectrometry results confirmed that the A₁ chain of SLT-1 binds to the ribosomal stalk proteins P0, P1, and P2. Moreover, the removal of the last 17 amino acids of either proteins P1 and P2 abolishes their interaction with the A₁ chain whereas P0 lacking this common C-terminus still binds to the A₁ domain. *In vitro* pull-down experiments using fusion protein-tagged C-terminal peptides corresponding to the common 7, 11, and 17 terminal residues of P1 and P2 confirmed that the A₁ chain of SLT-1 as well as the A chain of ricin bind to this common C-terminal peptide motif. In addition, this peptide was then shown to inhibit the

action of the A₁ chain of SLT-1 *in vitro*.

Conclusion: These results suggest a role for the ribosomal stalk in aiding the A₁ chain of SLT-1 and other structurally and functionally related RIPs, such as ricin, in localizing their catalytic domain near the site of depurination in the 28S rRNA. More importantly, a synthetic peptide corresponding to the C-terminus of the ribosomal stalk proteins was shown to inhibit the ribosome-inactivating function of the A₁ chain of SLT-1 *in vitro*, as measured by transcription/translation-coupled and cell-based cytotoxicity assays. These results suggest that this conserved peptide may prove to be a potential therapeutic against a broad range of RIPs.

48. Tumour-Targeted RIPs as Anti-Cancer Drugs

Melissa C. Cheung^{1,2}, Xin Wei², and Jean Gariépy^{1,2,3}. ¹Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario; ²Ontario Cancer Institute, University Health Network, Toronto, Ontario; ³Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada.

Purpose: The surface of cancer cells display arrays of receptors that can serve as targets in the development of diagnostic and therapeutic agents. Specifically, these surface markers may represent validated targets for drug discovery.

Methods: In an effort to distinguish surface signatures on normal and malignant cells, our group has constructed combinatorial protein libraries of the ribosomal-inactivating protein (RIP), that require cells to internalize these protein variants in order to cause cell death. Specifically, a combinatorial library of the cytotoxic domain (A chain) of Shiga-like toxin 1 (SLT-1) was created by inserting a random 7-residue long peptide within its structure. Thousands of A chain mutants were subsequently purified and screened for their ability to be internalized and to selectively kill human 518-A2 melanoma cells.

Results: This approach led to the identification of a cytotoxic variant (named SLT-1A^{IYSNKL}), which harbours a novel specific peptide ligand able to kill 518-A2 cells as well as several other human melanoma cell lines. Biodistribution and imaging studies of radiolabeled SLT-1A^{IYSNKL} in SCID mice bearing a human melanoma xenograft indicate that it readily accumulates at the tumour site.

Furthermore, the co-administration of SLT-1A^{IYSNKL} with dacarbazine (DTIC), a standard melanoma therapeutic, resulted in tumour regression and increased survival. SLT-1A^{IYSNKL} is stable in serum and binds to human melanoma 518-A2 cells with a dissociation constant of approximately 20 nM.

Conclusion: These results demonstrate the therapeutic potential of our cytotoxic variant, SLT-1A^{IYSNKL}, in the treatment of melanoma. Furthermore, this validates the screening of such combinatorial protein libraries as an approach for the identification of novel tumour biomarkers as well as cancer-targeted toxin therapeutics.

49. Tissue Drug Concentrations, Oxidative DNA Damage and Embryopathies in Acatalasemic and Catalase-Overexpressing Embryos Exposed in Utero to Phenytoin

Julia Perstin¹ and Peter G. Wells^{1,2}. Departments of ¹Pharmaceutical Sciences and ²Pharmacology and Toxicology, University of Toronto, Toronto, ON.

Teratogenicity of the antiepileptic drug phenytoin (5,5-diphenylhydantoin, Dilantin®) may be due in part to enhanced formation of reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂), which is detoxified by the antioxidative enzyme catalase. Although protein therapy with exogenous catalase reduces phenytoin teratogenicity in mice, little is known about the embryoprotective importance of the endogenous enzyme, which exhibits low activity during organogenesis. In this study we investigated the protective importance of constitutive catalase in catalase-deficient (acatalasemic) (C3Ga.Cg-Catb/J) mice and transgenic (C57BL/6 Tg(CAT)+/+) mice expressing human catalase in addition to murine catalase. Pregnant dams were given phenytoin, 65 mg/kg i.p., on gestational day (GD) 13 (GD 1 = plug), sacrificed 6 hr post injection and embryos were explanted and analysed for oxidative DNA damage in the form of 8-oxoguanine using high-performance liquid chromatography (HPLC) with electrochemical detection. Phenytoin did not increase DNA oxidation or cause embryopathies in either acatalasemic embryos or their wild-type controls. Conversely, transgenic embryos overexpressing catalase exhibited lower constitutive levels of DNA oxidation than their wild-type controls, and unlike wild-type controls exhibited increased DNA oxidation when exposed to

phenytoin. Teratological studies in this strain are ongoing. Phenytoin concentrations in fetal brain determined by HPLC were about 60% higher than those in fetal liver, which were similar to those in both maternal liver and brain. These results suggest that the acatalasemic mouse strain may be relatively resistant to phenytoin-enhanced ROS formation and teratogenicity, requiring further optimization of the treatment regimen to evaluate the embryoprotective role of endogenous catalase. The lower constitutive and phenytoin-enhanced DNA oxidation in catalase overexpressing embryos suggests a protective role for endogenous catalase, although the teratological relevance remains to be determined. The increased concentration of phenytoin in fetal brain compared to fetal liver and maternal tissues may explain the high frequency of neurodevelopmental deficits compared to other anomalies caused by this drug, and raises interesting questions as to the mechanisms underlying this selectivity.

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50. Hepatic Expression of ABC Drug Transporters in Rodent Models of Inflammation During Pregnancy

Vanja Petrovic and Micheline Piquette-Miller.
Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.

Purpose: While various rodent models of inflammation and infection have been established, few have focused on using pregnant rats. Pregnancy induces a number of physiological changes which may affect the response of these rats to an infectant. Our objective was to compare three different inflammation models using pregnant rats in order to determine the best one for use in future pharmacokinetic studies. We examined the models in terms of their effect on the expression of several key hepatic drug transporters and the metabolizing enzyme Cyp3a, as these genes are known to be affected by the inflammatory response and also have many clinically relevant substrates.

Methods: Pregnant SD rats (G17-18, n=3-6/group) were administered single i.p. doses of the following infectants: bacterial endotoxin (LPS, 0.1 - 1.0 mg/kg), polyinosinic:polycytidylic acid (poly I:C, 0.75 - 5.0 mg/kg), or interleukin-6 (IL-6, 1 µg/rat). Control pregnant rats received saline. Animals were sacrificed 6-24 hrs later and hepatic mRNA levels of P-glycoprotein (Mdr1a and Mdr1b), Mrp2, Bcrp,

Oatp2, and Cyp3A were measured via real-time PCR.

Results: At the given doses, LPS and poly I:C models were the only to have a significant effect on hepatic drug transporter and Cyp3a expression levels as compared to controls. No significant differences were observed with the IL-6 model at the given dose. Expression profiles of mRNA levels were similar between the LPS and poly I:C models, with significant downregulation ($p < 0.05$) of all examined genes except for Mdr1b, which was significantly induced. However, LPS was more toxic for pregnant rats than poly I:C was.

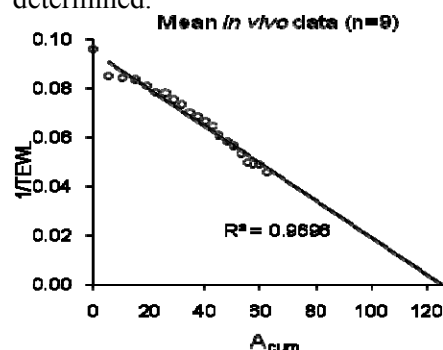
Conclusion: Both, LPS and poly I:C-induced inflammation in pregnant rats are effective for studying the impact of inflammation on hepatic drug transporters and drug disposition of their substrates. From our observations, poly I:C was safer for use in pregnant rats as it resulted in less mortalities. IL-6 dosing would have to be optimized in order to provide an effective model, but this may be costly as cytokines are an expensive medium.

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51. Quantitation of Stratum Corneum Depth in the Skin Surface Biopsy by Tape Stripping

JE Grice¹, L Stewart^{2,3} and MS Roberts¹.
¹Therapeutics Research Unit, School of Medicine, University of Queensland, Australia; ²Faculty of Pharmacy, University of Manitoba, Winnipeg, Canada; ³Visiting Scholar, University of Queensland.

Purpose: The skin surface biopsy by tape stripping allows successive sampling of the stratum corneum (SC) at increasing depths. However, SC thickness varies between subjects and sites and the amount of SC material removed varies between strips. This work shows a method by which the relative SC depth and amount removed with each strip can be determined.



Methods: A site on the inner volar forearms of nine normal human volunteers was stripped 20 times with 14 mm CuDerm discs. After each strip, transepidermal water loss (TEWL) was measured at the site with a Tewameter (Courage-Khazaka). The absorbance of each strip was read at 850 nm (SquameScan 850). These procedures were repeated using excised human skin from a single female donor (11 replicates). Recognizing that Fick's 1st Law describes water diffusion through the SC membrane, a linear relationship exists between the inverse of TEWL and the thickness of the membrane after each strip. The thickness of the unstripped SC is given by the x-axis intercept of such a plot. As absorbance (A) is proportional to the thickness of the absorbing material (Beer-Lambert Law), the cumulative absorbance (A_{cum}) of the material on the strips can be substituted for thickness to obtain a similar relationship. This allows us to obtain a depth at each tape strip relative to the total depth. It also allows comparison between individual subjects.

Results: The predicted theoretical relationship between $(TEWL)^{-1}$ and A_{cum} was verified experimentally for the human subjects (see figure). The mean percentage of SC removed after 20 strips was 49.2% (%CV=21.6). There was significant variability in the SC thickness removed by the 20 strips (represented by A_{cum} values; mean=62.5, %CV=26.2). The same linear relationship was also verified for excised skin ($R^2=0.9916$). The percentage of SC removed by 20 strips (mean=82.6, %CV 3.6%) was greater than seen for *in vivo* skin. As well, a greater absolute amount of SC was removed from the excised skin (mean A_{cum} = 96.72, compared to 62.5 for *in vivo* skin).

Conclusions: We demonstrated a simple method for determining relative depth in SC after tape stripping. While comparable methods were previously applied to *in vivo* skin, we showed for the first time that theoretical predictions also hold true *in vitro*. Inter-individual differences may explain the differences between *in vivo* skin and the single sample of excised skin, or they may reflect different rates of diffusion in non-living tissue.

Reference: Herkenne, C. et al (2008) *In vivo* methods for the assessment of topical drug bioavailability Pharm Res 25(1):87-103.

52. A Cree Anti-Diabetic Botanical Alters Gene Transcript Changes in Caco-2 Cells

Carolina Ogradowczyk^{1,2,3}, Charlotte McDonald^{2,3}, Jason Popescu¹, Asim Muhammad^{2,3}, Brendan Walshe-Roussel^{1,2,3}, Ammar Saleem^{2,3}, Brian C. Foster^{2,3,4}, John T. Arnason^{1,2,3}. ¹Ottawa-Carleton Institute of Biology, University of Ottawa, Ottawa, Ontario, Canada; ²Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa, Ottawa, Ontario, Canada; ³CIHR Team in Aboriginal Anti-Diabetic Medicines, ⁴Office of Science, Therapeutic Products Directorate, Health Canada, Ottawa, Ontario, Canada.

Purpose: To evaluate a Cree botanical, AD09, used by the Cree of Eeyou Istchee to treat symptoms of type II diabetes (T2D), for its role in altering gene transcript changes in human Caco-2 intestinal cells. Also, to undertake a quantitative comparison of the phytochemical profiles of ethanol (EtOH) and traditional water (DW) extracts to determine the relevance of using an EtOH extract in laboratory studies.

Methods: Human Caco-2 intestinal cells were exposed to AD09 at a concentration of 100 μ g/mL for a period of 4 and 24 hrs, the RNA was extracted, and microarray experiments were performed using human 19K cDNA arrays to establish gene changes with the extract versus 0.1 % DMSO control. Furthermore, cells were exposed to compound M, a major component of AD09 for 4 hours, at a concentration of 8.8 μ g/mL, and microarray experiments performed. Both extracts were analyzed by HPLC coupled with diode array detector and evaporative light scattering detector.

Results: Microarray experiments for AD09 and compound M for 4 hrs revealed no statistically significant gene transcript changes. However, the 24-hr exposure to AD09 yielded 304 downregulated mRNAs ($p<0.05$) with a fold-change greater than 1.5 with significant downregulation of key transcription factors and members of different signaling pathways. A phytochemical evaluation yielded many similarities between the extracts in the different markers examined. Only slight differences were observed in the polar and non-polar regions of the chromatograms.

Conclusions: AD09 did not cause transcript changes of the cytochrome P450s which are usually altered in the intestinal model of Caco-2 cells upon exposure to a xenobiotic, signifying that the botanical may be quite safe to use especially with

co-administered therapies. However, there does appear to be an overall shutdown of transcriptional machinery at 24 hrs which may be associated with the decreased amounts of transcription factors as well as signaling molecules. Furthermore, since both extracts have similar chromatographic profiles, the EtOH extract can be used in a laboratory setting.

53. Phosphorylation Levels of Extracellular Signal-regulated Kinases (ERK) Determine the Sensitivity of Hepatic Stellate Cells to Staurosporine-Induced Apoptosis

Jianghong Fan¹, Qiaomei Dai², Frank J. Burzynski¹, Gerald Y. Minuk³, Yuewen Gong^{1,3}. ¹ Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada; ² The Second Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang Province, China; ³ Section of Hepatology, Department of Internal Medicine, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.

Purpose: Hepatic stellate cells (HSCs) are the principal cells responsible for the development of hepatic fibrosis and cirrhosis. During the fibrotic process, HSCs undergo proliferation and transdifferentiation from a quiescent to myofibroblast-like phenotype. The fate of myofibroblast-like HSCs includes apoptosis or reversion back to a quiescent phenotype. The mechanism involved in the apoptotic process has yet to be determined.

Methods: By employing four rat HSC cell lines (CFSC-8B, -2G, -3H and -5H), we documented the expression of extracellular signal-regulated kinases (ERK) by Western blot analyses and cell apoptosis by flow cytometry.

Results: Each HSC cell line had a distinct morphology, consistent with the expression of alpha smooth muscle actin (α -SMA) such that CFSC-8B cells had the highest α -SMA expression. Although all four cell types expressed similar levels of ERK1/2, phosphorylation levels were significantly higher in CFSC-8B and CFSC-2G than CFSC-3H and CFSC-5H cells. When CFSC-8B cells (high ERK1/2 phosphorylation) and CFSC-5H cells (low ERK1/2 phosphorylation) were then employed to examine staurosporine-induced apoptosis, CFSC-8B cells were found to be significantly more sensitive. Moreover, staurosporine further increased ERK1/2 phosphorylation in both cell lines.

Conclusion: ERK1/2 phosphorylation in hepatic

stellate cells determines the sensitivity of these cells to staurosporine-induced apoptosis.

(This study was supported by the Canadian Institute of Health Research.)

54. Investigating Insulin and Glucocorticoid Signaling in LXR-Deficient Mice

Jesse Goldmacher¹, Monika Patel² and Carolyn L. Cummins². ¹ University of Western Ontario, Faculty of Medical Sciences, London, ON; ² University of Toronto, Leslie Dan Faculty of Pharmacy, Toronto, ON.

Purpose: Glucocorticoids such as dexamethasone (DEX), are widely prescribed immunosuppressant drugs. However, their long term use is limited by their undesirable effects of elevated blood sugar levels which lead to insulin resistance and Type II Diabetes. Thus, finding a way to use glucocorticoids to suppress the immune system, without affecting blood sugar levels is paramount. Liver X Receptors (LXRs), which respond to cholesterol metabolites, have recently been implicated in glucose homeostasis. Findings by Cummins et al. (*unpublished*), showed that Liver X Receptor double knockout (LXR α/β -/- DKO) mice are resistant to glucocorticoid-induced hyperglycemia, while remaining sensitive to immunosuppression. The current study attempts to illustrate the mechanism by which these DKO mice remain resistant to hyperglycemia.

Methods: *In vitro* studies were conducted on H4IIE cells to determine the effects of DEX on insulin signaling by looking at a well known insulin signaling protein (P-Akt) via Western Blot analysis. Also, *in vivo* studies on wild type (WT) and DKO mice given either vehicle or DEX with or without insulin were conducted to see the difference in response between genotypes.

Results: Insulin increased cytoplasmic P-Akt levels, and the co-incubation with DEX reduced P-Akt induction by 16%. Basal cytoplasmic P-Akt levels were lower in the DKO mice. Additionally, DKO mice had a 51% increase in cytoplasmic P-Akt/Akt ratio with insulin treatment compared to an 8% increase in the WT mice. However, with co-administration of DEX, DKO mice had a similar response to WT mice. It was observed that glucocorticoid receptor (GR) nuclear translocation, a necessary step in glucocorticoid signaling, may be reduced in DKO mice.

Conclusions: Differences in insulin signaling and

GR translocation may point towards the mechanism of resistance to glucocorticoid-induced hyperglycemia in DKO mice and ultimately a direction for minimizing the adverse effects of immunosuppressive therapy with glucocorticoids.

Drug Delivery and Pharmaceutical Technology

55. Dielectric Analysis of Drugs Reveal Crystalline and Amorphous Content and Transition Temperatures

Manik Pavan Maheswaram^[1], Alan Riga^[1, 2] and Kenneth Alexander^[2]. ^[1]Department of Chemistry, Cleveland State University, Cleveland, Ohio, USA; ^[2] Division of Industrial Pharmacy, Department of Pharmacy Practice, College of Pharmacy, University of Toledo, Toledo, Ohio, USA.

Purpose: In pre-formulation development solid state transitions as well as melting and crystallization are critical physical properties. Differential Scanning Calorimetry (DSC) and Dielectric Analysis (DEA) can easily differentiate morphological and thermodynamic transitions in drugs as well as their crystalline-amorphous content. The DSC crystalline content is based on the melting endotherm of the drug and its ability to recrystallize exothermically. The content is determined from a number of heat and cooling cycles at a constant heating rate to evaluate the drugs ability to recrystallize. The DEA electrical conductivity analysis clearly and repeatably differentiates the solid crystalline low conductivity level and the high conductivity amorphous liquid. The DSC sets the transition range and the DEA conductivity establishes the content.

Methods: DSC is set at a standard 10°C/min heating rate-cooling rate, sample size 3-5 mgs, in nitrogen atmosphere with a standard aluminum pan and lid. The DEA is set at a standard 10°C/min, sample size 10 mgs, in a nitrogen atmosphere with a gold ceramic interdigitated electrode. The drugs evaluated were from a US Pharmacopoeia set of melting point standards.

Results: The DSC results are compared to the DEA conductivity amorphous and crystalline content identified below the DSC melting temperature for the standard drugs. To establish a structure property relationship where the crystalline character is

recorded by PXRD and compared to the content by DEA and DSC. The Active Pharmacy Ingredients (APIs) evaluated included Acetanilide, Acetophenetidin, Sulfapyridine, and Caffeine.

Conclusions: There was a fair-good agreement between the DSC crystalline melting and recrystallization and the solid state DEA conductivity method. The amorphous and crystalline content of the APIs is clearly and repeatably determined in the DSC or DEA cyclic heating and cooling of the drug. The transition from crystalline solid to liquid amorphous drug was accompanied by a conductivity change from ca. 10^{-1} pS/cm to 10^7 pS/cm. Reheating the sample API produced a decrease in crystalline content resulting in a 90% decrease for caffeine and a 18 % decrease for acetanilide.

56. Improved Thermal Mechanical Method for Evaluating Drug Delivery of Tablets and Capsules

Visweswararao Badipatla^[1], Indika Perera^[1], Manik Pavan Maheswaram^[1], Alan Riga^[1, 2] and Kenneth Alexander^[2]. ^[1]Department of Chemistry, Cleveland State University, Cleveland, Ohio, USA; ^[2] Division of Industrial Pharmacy, Department of Pharmacy Practice, College of Pharmacy, University of Toledo, Toledo, Ohio USA .

Purpose: In 2009 the United States Pharmacopoeia (USP) test for tableted drug disintegration is limited and does not specify the initial disintegration time. This national test gives limited information on the disintegration process of tablet and capsule drugs. Thermal Mechanical Analysis (TMA) has been developed to now measure the rate and initial time of drug disintegration. The TMA monitors the physical dimension of the formulated drug as a function of time, temperature, applied stress and pH. The results of this new test will meet USP specifications. This method can be used to measure the expansion, or swelling of the formulated tablet. It can also monitor the shrinkage or drug disintegration in a specified fluid.

Methods: The focus of this study is creating an efficient and precise method to measure the drug delivery of solid dose tablets and capsules. The precision of the method along with the effect of pH and temperature on the rate of delivery was determined. The drugs studied were Femhrt and Aspirin tablets as well as Amoxicillin Capsules. The TMA measures the dimensional stability of the formulated drug as it is immersed in various

solutions at 25 or 37°C.

Results: Graphical representations of these dimensional changes over time were created and compared. Drug delivery in a specific solution was measured by UV Analysis for the active pharmaceutical ingredient. Temperature decreased the disintegration time and increased the rate (mm/min). The amoxicillin 500 mg capsule disintegrated by first absorbing the solvent water and softened. Then the 2nd step was the rounded ends collapsed allowing the drug to be released to the solution. The increased temperature shortened the capsule dissolving time. For the drugs studied pH did not have an appreciable effect on the rate of disintegration.

Conclusions: Tablets or capsules disintegrate and release the drug while it structurally is falling apart. Some tablets swell and allow the active ingredient to be released. Some tablets swell and then disintegrate either rapidly over a period of time. This TMA method distinguished clearly the orally disintegrating drug (Olanzapine® in 18 seconds), the drug delivery to the stomach, e.g. Femhrt® (1-20 minutes) and those drugs that are bound for the intestines (Ritalin® in >39 minutes and Abilify® >60minutes).

57. Development of a Polymer-drug Conjugate based Micelle System for the Systemic Delivery of Docetaxel to Tumors

Andrew Mikhail^{1,2}, Christine Allen^{1,2,3,4*}

¹Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy; ²Institute of Biomaterials and Biomedical Engineering; ³Department of Chemical Engineering and Applied Chemistry, Faculty of Applied Science and Engineering; ⁴Department of Chemistry, Faculty of Arts and Science, University of Toronto, Toronto, Ontario, Canada.

Purpose: Block copolymer micelles are capable of improving the therapeutic efficacy of hydrophobic and highly toxic chemotherapeutic agents. However, their role as true site specific drug delivery vehicles has been limited by poor drug retention upon i.v. administration. In this study, docetaxel (DTX) was conjugated to the core of poly(ethylene)oxide-*block*-polycaprolactone (PEG-*b*-PCL) micelles in order to increase drug-core compatibility for loading and retention of unbound DTX. The effect of drug conjugation and changes to the length of the hydrophobic block were explored with respect to the state of the micelle core, micelle morphology and

stability.

Methods: Characterization of copolymer-drug conjugates was performed using ¹HNMR and GPC. The amount of DTX conjugated to the copolymer was determined using HPLC. Micelles were generated by the evaporation method and their morphology and stability were observed using TEM and dynamic light scattering, respectively.

Results: A high conjugation efficiency was achieved for the conjugation of DTX to the copolymer. Various morphologies were observed for the copolymer aggregates in solution while aggregates of the copolymer-drug conjugate were entirely spherical. Micelles were stable in solution for at least one week at a size of ~10nm. Conjugation of DTX to the copolymer resulted in a 175 fold increase in DTX solubility.

Conclusions: Docetaxel has been successfully conjugated to the core of PEG-*b*-PCL copolymer micelles resulting in a significant improvement in the drug's water solubility. The superior stability and drug loading capacity of this micellar delivery system make it a promising drug delivery platform for further development.

58. New Electrokinetic Method for Characterizing Polyelectrolyte Gels for Drug Delivery

Beibei Qu, Ping I. Lee. Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada.

Purpose: Polyelectrolyte gels have been studied as stimuli-responsive drug delivery systems as they are responsive to environmental stimuli such as pH, ionic strength and electrical potential. Charged groups in the gel phase play an important role in affecting the diffusional release of an entrapped ionic drug when there are interactions with fixed charge groups in the gel phase. However, the current microslit method for characterizing charge properties of a thin gel cannot be easily applied to gels of pharmaceutical interest. As a result, more rigorous methods for quantifying such charge effect have been lacking. In the present work, we characterize the charge effect of gelatin gels using an improved rotating disc method, which is much simpler than the microslit method. A more accurate model taking into account the effect of surface conductivity factor is also proposed.

Methods: A gelatin gel sample (10% pig gelatin crosslinked with 2% glutaraldehyde) formed in an

insulated dish (diameter 1.1cm, depth 0.5cm) was attached to a spindle with the gel surface facing downward. The spindle was immersed in a NaCl solution and rotated at a fixed rate (0 to 2500 rpm). Two Ag/AgCl electrodes connected to a Keithley Electrometer (Model 614) were placed, one at the center of the dish 0.5 mm from the sample surface and the other far away from the sample. The equivalent surface potential and charge density of the polyelectrolyte gel were calculated from the measured streaming potential based on a developed rotating disc model.

Results: The gelatin gels were negatively charged under the experimental pH of 5.8 (0.04 mM and 0.1mM NaCl solution). An electrokinetic model for such rotating polyelectrolyte gel disc taking into account the effect of surface conductivity factor was established. The surface conductivity of the gelatin gel from the rotating disc method was calculated to be about 1.35×10^{-5} S, and the volume charge density was estimated to be 0.019M, which is close to the results obtained from titration or conductivity measurements. The surface potentials of this gelatin gel were calculated to be 131mV and 108mV in 0.04 mM and 0.1mM NaCl solution, respectively.

Conclusions: Our current results indicate that the surface conductivity of a polyelectrolyte gel in the rotating disc method cannot be ignored and the improved rotating disk method can be conveniently set up and applied to quantify the volume charge density and surface potential of polyelectrolyte gels for drug delivery applications.

59. Peptide-based Delivery of Therapeutic Molecules into Cells

Amaalia Emma Broad¹, Andrew Jeffrey McCluskey¹, Jean Gariépy^{1, 2}. ¹ Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada; ² Ontario Cancer Institute, University Health Network, Toronto, ON, Canada.

Purpose: The delivery of biomolecules into living cells by cell penetrating peptides (CPPs) has proven to be a novel therapeutic strategy. Recent studies suggest endocytosis as the internalization method of CPPs and their cargo. However the efficiency of CPPs is hindered by: entrapment into vesicles, degradation, recycling out of cells, and consequently limited delivery into the cell cytoplasm and nucleus. To evade these barriers, we are investigating small protein domains that are able to exit from vesicular

compartments. One such vesicle-to-cytosol translocation domain (VCTD) was derived from a bacterial protein and fused to a CPP with the aim to improve the delivery of cargos (protein and nucleic acids) to the cytosol.

Methods: HeLa and 293 T cell lines were treated with recombinant CPP-VCTD constructs either fused to a protein cargo (eGFP) or complexed with nucleic acids (luciferase and eGFP reporter plasmids). Live cell imaging and FACS analysis were used to visualize and quantify internalization and escape of CPP-VCTD-eGFP constructs. In order to quantify internalization and escape of CPP-VCTD complexed with plasmid DNA (pGL2 or pEGFP-N1), the expression of a reporter gene (luciferase or eGFP) was monitored by measuring relative light units per total mg protein, FACS analysis, and live cell imaging.

Results: A series of recombinant protein constructs containing the VCTD fused to a CPP and in some cases to eGFP have been cloned, expressed, and purified. Confocal microscopy and flow cytometry results show internalization of such constructs into cells, and endosomal escape/cytosolic dispersion of the eGFP-labeled protein. In addition to the delivery of fused-protein cargos, functional assays determined that our CPP-VCTD constructs can act as carriers to import cargos such as DNA plasmids, which readily form complexes in solution.

Conclusion: This translocation domain may prove useful for drug delivery, particularly for protein therapeutics, siRNA delivery, and vaccine formulations.

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60. Biochemical Evaluation of Dermal Reconstitution with Hyaluronan

Loida Oruña^{1,2}, Gabriel Coto³, Guillermo Lago³. ¹State Center Quality and Control of Drug, Havana City, Cuba; ² Medicine Latinoamerican Faculty, Havana City, Cuba; ³ Placental Histotherapy Centre, Havana City, Cuba.

Purpose. To assess the dermal reconstitution in open wounds treated with Hyaluronan jelly (HA) biochemical indicators, prolin, hydroxyprolin, glycine, hystidine, lysine and total amino acid content in dermis were determined.

Methods: Jelly containing 0 (placebo), 2, 4 and 8% HA were applied to excisional wound in the similar area of dermis of rat skin and their effect assessed on

5th and 7th days. Acid hydrolysis were made with the objective of quantified the alpha amino acid groups from nitrogen content and total amino acid with.

Results: The amino acids in the neformatted matrix in rats after 7 days of the treatment (mean $\pm$ SE of relative area of the cromatograms) was:

Amino acid	Placebo	Spontaneous Control	Positive Control	2% HA Jelly	4%HA Jelly	8% HA Jelly
OH Proline	7.24 $\pm$ 0.81	7.52 $\pm$ 0.69	6.37 $\pm$ 0.56	4.61 $\pm$ 0.32	10.33 $\pm$ 0.87	13.39 $\pm$ 0.88
Proline	29.68 $\pm$ 1.12	10.38 $\pm$ 0.84	15.94 $\pm$ 0.94	29.33 $\pm$ 1.07	153.95 $\pm$ 9.32	163.68 $\pm$ 9.36
Glycine	120.91 $\pm$ 4.23	125.60 $\pm$ 5.21	206.02 $\pm$ 9.97	138.46 $\pm$ 6.22	309.87 $\pm$ 10.29	401.76 $\pm$ 10.96
Hystidine	18.41 $\pm$ 0.96	13.05 $\pm$ 0.78	43.30 $\pm$ 1.82	54.38 $\pm$ 2.01	43.22 $\pm$ 1.80	47.13 $\pm$ 1.92
Lysine	89.97 $\pm$ 3.20	64.75 $\pm$ 2.42	81.39 $\pm$ 2.91	115.29 $\pm$ 4.11	111.01 $\pm$ 3.86	125.54 $\pm$ 4.76

The effect of the treatments coincided with matrix histology of the collagen fibres. The alpha amino nitrogen and glycine quantitative values indicate collagen synthesis and dermal reconstitution. Hydroxyproline value, a marker of the effectiveness, demonstrated adequate response.

Conclusion: Correlation between the biochemical and histological results in the dermis indicate that the 4% HA is the most effective among the examined formulations in terms of dermal reconstitution.

61. Formulating Poorly Soluble Drug for Ocular Delivery

Kay Koch-Gaynor,¹ Mehran Maleki¹.
¹GlaxoSmithKline Canada Inc., Pharmaceutical Development, Mississauga, ON, Canada.

Purpose: Oral dosage form is by large the most common form for drug delivery; however depending on the target and disease area this may not be the best route for administering drug to the eye. The objective of this research was to explore a polymer-based formulation and show its feasibility for delivery drug to the eye with sustained-release action. The work was carried out using compound X, a poorly soluble compound.

Methods: Using a full factorial DOE design experiment, and by varying the drug loads and the polymer A and B in the matrix, the formulations were tuned to deliver microgram per day drug concentration, suitable for ocular delivery. Due to poor aqueous solubility of compound X, we have used the hydrochloride salt version with particle size reduction to aid drug solubility in the formulation design. XRPD was used to characterize the formulations and monitor for form conversion. An *in vitro* dissolution methodology was employed to

screen the formulations; two formulations were progressed in a 12-week preclinical ocular PK study.

Results: *In vitro* drug release data exhibited characteristic profiles of sustained-release action. Multiple parameters were controlling the formulation drug release rate; release rate was found directly proportional to the drug load and the drug to polymer ratio in the formulation, but inversely proportional to the ratio of polymer A and B. It was found that the API has a tendency to convert back to the parent free base, which limits the solubility of the drug in dissolution testing; this was shown in XRPD. Formulations in preclinical PK study confirmed ocular exposure after 5 days, 40 days and 82 days.

Conclusion: Polymer-based formulations are a viable option for delivery poorly soluble drug for sustained-release action to the eye; drug diffusion was evident throughout the ocular tissues.

62. Development of a Particle Size Reduced Formulation

Kai Zhang¹, Andrea Toth¹, Kay Koch-Gaynor¹, Mehran Maleki¹.
¹GlaxoSmithKline Canada Inc., Pharmaceutical Development, Mississauga, ON, Canada.

Purpose: Poorly soluble drugs pose significant challenges to oral exposure. A low solubility NCE compound under development has a further challenge of a cost of goods target that restricts it to simple and conventional formulations that can be manufactured at relatively low cost.

Methods: Several approaches for enhancing drug solubility in the final dosage form have been investigated. Among the options were API particle size reduction, the use of surfactants such as sodium lauryl sulfate, and cyclodextrin complexation. The formulations in development included (1) a tablet formulation consisting of particle size reduced API with conventional tableting technology (preferred dosage form); (2) a suspension of particle size reduced API (particle size range #1) in water with SDS and HPMC; (3) a suspension of particle size reduced API (particle size range #2) in water with Tween 80 and HPMC; (4) a simple granulation of particle size reduced API with cyclodextrin. All four formulations were tested in a pre-clinical PK study to predict the exposure in humans, at two dose levels (low and high).

Results: The tablet formulation (1) performed less favourably compared to the other three alternatives.

The pre-clinical PK study results at the lower dose level showed that the AUC for formulation (2) was two-fold of that obtained for the tablet formulation (1), while formulations (3) and (4) were about six to seven-fold higher than for the tablet. At the higher dose level, the three alternative formulations all produced two to three fold higher AUC results than the tablet formulation.

Conclusion: The outcome of the preclinical study supported the progression of a formulation that contained particle size reduced API (particle size range #2) and supplied as a powder in capsules. The formulation has been progressed to human volunteer studies.

63. Transdermal Pharmacokinetics of Repellent DEET and Sunscreen Oxybenzone in Rats

Daryl Fediuk, Xiaochen Gu. Faculty of Pharmacy, University of Manitoba, Winnipeg, MB, Canada

Purpose: Repellent DEET and sunscreen oxybenzone (OBZ) are capable of transdermal permeation and systemic absorption. Correlation between skin/tissue disposition and pharmacokinetics of DEET and OBZ has not been fully elucidated. This study investigated the pharmacokinetics of transdermal DEET and OBZ in rats.

Methods: DEET (100 mg/kg) and OBZ (40 mg/kg) were topically applied to male Sprague-Dawley rats, either alone or in combination. Blood samples were collected for 24 hours after the administration. Livers and kidneys were harvested at the end of the study. Concentrations of DEET and OBZ were analyzed using a validated HPLC method. Pharmacokinetic analysis was performed using WinNonlin[®] software.

Results: Upon reaching maximal blood concentration (T_{max}) at 90 minutes, DEET plasma concentrations quickly decreased with a mean absorption time (MAT) and an elimination half-life ($t_{1/2}$) of 127.9 ± 26.5 min (Mean $\pm$ SEM, $n=5$) and 361.0 ± 50.2 min, respectively. The combined application produced a faster MAT (73.1 ± 30.4 min) and a shorter $t_{1/2}$ (202.4 ± 19.6 min). Significant amounts of DEET were detected in rat tissues; combined use with OBZ enhanced DEET deposition in the liver (36.1 ± 2.7 ng/g vs. 17.6 ± 2.9 ng/g), but resulted in no difference in the kidney (9.9 ± 1.6 ng/g vs. 10.1 ± 1.8 ng/g). With a T_{max} of 150 minutes, MAT and $t_{1/2}$ of OBZ for combined use (136.6 ± 42.7 min/ 449.3 ± 18.3 min) were shorter than those of

single use (208.2 ± 45.1 min/ 741.6 ± 73.4 min). Considerable tissue distribution of OBZ after 24 hours indicated that OBZ had not been fully metabolized; combined use produced higher deposition than single use in both liver (60.5 ± 4.2 ng/g vs. 39.7 ± 2.2 ng/g) and kidney (89.4 ± 3.3 ng/g vs. 74.4 ± 6.9 ng/g). Significant differences in $t_{1/2}$ and liver concentrations were found for DEET and OBZ between the two applications ($p < 0.05$).

Conclusion: DEET and OBZ demonstrated prompt percutaneous absorption and extensive distribution following transdermal applications in rats. Further evaluation is required to correlate pharmacokinetic parameters to toxicological profiles to understand the benefits/risks of concurrent use of repellent and sunscreen preparations.

64. Biocompatibility and *in vitro* Efficacy of Injectable Polymer-lipid Blends for Localized Taxane Delivery

R. De Souza¹, P. Zahedi¹, C. Quach¹, C. Allen¹, M. Piquette-Miller¹. ¹Department of Pharmaceutical Sciences, University of Toronto, Toronto, Canada.

Purpose: Biocompatible injectable drug delivery systems are desirable due to minimal invasiveness and improved patient compliance. The present work examined the biocompatibility of injectable chitosan-phospholipid blends, PoLi_{gel}-LA and PoLi_{gel}-LCl.

Methods: *In vitro* cytotoxicity was evaluated by exposing L929 and HeLa cells to varying amounts (0.0013 to 6.25 μ g/mL) of PoLi_{gel}-LA or PoLi_{gel}-LCl for 48 hours. Cytotoxicity was measured by the MTT assay. *In vivo* biocompatibility was investigated by subcutaneous and intraperitoneal (I.P.) injections of the blends into healthy CD-1 mice. Toxicity assessment was done by histological examination of tissues and measurements of plasma ALT and IL-6 levels. To assess suitability of the system for drug delivery in cancer therapy, PoLi_{gel}-LA was loaded with docetaxel and was exposed to SKOV3 and A2780 cells at varying concentrations (0.16 to 5.00 μ g/mL) to assess growth inhibition.

Results: Both blends resulted in acceptable biocompatibility *in vitro*, although greater viability was seen with PoLi_{gel}-LA. *In vivo*, the PoLi_{gel}-LA blend caused no local or systemic toxicities over a four-week period, while the PoLi_{gel}-LCl blend performed poorly subcutaneously and thus was not tested further. Mice injected I.P. with PoLi_{gel}-LA showed no physical or behavioural alterations, and

body weight changes did not differ from control animals. Histology of relevant tissues showed unaltered morphology. Plasma interleukin-6 levels in mice injected with PoLi_{gel}-LA did not differ from levels of control animals (6.91 ± 3.61 pg/mL versus 6.92 ± 5.02 pg/mL, respectively). Biodegradation occurred progressively, with 7.4 ± 5.02 % of the original injected mass remaining after four weeks. PoLi_{gel}-LA loaded with docetaxel showed comparable biocompatibility to drug-free PoLi_{gel}-LA, with 95% of animals treated I.P. showing no evidence of peritoneal inflammation or irritation. The remaining 5% of animals showed only minimal abdominal swelling. *In vitro*, dose and time-dependent activity of docetaxel-loaded PoLi_{gel}-LA was observed, effectively inhibiting proliferation of SKOV3 and A2780 cells.

Conclusion: Results obtained herein establish the injectable PoLi_{gel}-LA as biocompatible and indicate its potential for localized delivery of anticancer agents such as docetaxel.

65. Development, Optimization and Evaluation of Diclofenac Loaded Flexible Liposomes

Amit Bhatia, Basant Amarji, Kaisar Raza, Bhupinder Singh, O P Katare. Drug Delivery Research Group, University institute of Pharmaceutical Sciences – UGC Centre for Advance Study, Panjab University, Chandigarh, India.

Purpose: Non-steroidal anti-inflammatory drugs (NSAIDs) are the “Gold Standards” in the treatments of various pain and inflammation related disorders like arthritis. However, oral administration of NSAIDs and use of newer selective COX-2 NSAIDs known to induce severe intolerance. Thus, the present work aims to develop and evaluate a novel carrier for targeted epicutaneous delivery of a potent NSAID, diclofenac. Flexible lipid vesicles (FLVs) are new generation liposomes designed to deliver drug to deep-seated inflamed and painful locations when applied topically.

Methods: Various FLV formulations were prepared to select the suitable material (lipid & edge-activator) and their levels. Systematic optimization studies were carried out, using 3^2 factorial design, to select the optimized FLV composition with reference to drug pay-load /entrapment, flexibility, vesicle count, turbidity, drug-leakage (stability), permeation and deposition of drug in skin. The prepared vesicular systems were characterized for various formulation attributes e.g., morphology,

micromeritics, physical stability and thermal properties. The pharmacodynamic activity of final optimized formulation was evaluated using carrageenan induced rat paw edema model along with radiographic analysis.

Results: The optimized and selected vesicular systems consisting of saturated phospholipid and sorbitan monooleate showed highest drug pay-load, maximum flexibility, highest vesicle count, lowest drug-leakage, higher drug permeation and drug deposition vis-a-vis other FLVs. While, thermographic, FT-IR and XRD analysis of FLVs, indicates the favorable packing arrangement between lipid and span consequently, fluidization in vesicle-lamellae, as revealed earlier in flexibility study. The drug permeation and skin-deposition from developed FLVs were found to be higher compared to liposomes and other tested formulations. The *in-vivo* pharmacodynamic result was found to be in agreement with the *ex-vivo* findings as FLVs have shown faster onset and prolonged anti-inflammatory effect, while radiographic analysis ratifies the above result. The results of dermal irritancy test along with histopathological study confirm the safety of diclofenac-loaded FLVs formulation as this was found to be devoid of any skin irritation reaction.

Conclusion: The results of present study demonstrated superior efficacy and safety of topically applied diclofenac-loaded flexible lipid-vesicles. Hence, it can be concluded that diclofenac in FLVs can be a very good formulation for management of various pain and inflammation related ailments including osteoarthritis.

66. The Biopharmaceutical Classification System: Can it Help Predict Bioequivalence Outcome? A CRO Retrospective Analysis

Stephane Lamouche, Hélène Lenard, Erik Shink, and Mario Tanguay. Anapharm, Ste-Foy, Québec, Canada.

Purpose: The biopharmaceutical classification system (BCS) is a scientific framework that categorizes drug substances into four classes according to their aqueous solubility and intestinal permeability. The purpose of this study was to conduct a retrospective analysis to determine if the BCS may help predict *in vivo* bioequivalence (BE) outcome. Other cofactors (i.e., fast vs. fed, low vs. highly variable drug) were also examined.

Methods: 918 BE studies (FDA, EMEA, HPFB)

with non-complicated IR oral dosage forms, representing 178 different compounds, were included in a descriptive analysis. Assignment of drugs to their predicted BCS class was done according to readily available databases and information from the literature. A logistic regression analysis was performed to assess the probability of meeting FDA's (most restrictive) BE requirements. The regression model was constructed from 524 studies (validation was performed with 168 studies), all taken from the original data set.

Results: Our descriptive analysis showed that overall, BE failure rate was generally low and similar (~11%) for highly soluble compounds (BCS I and III). Solubility appears to be the most discriminating factor with regards to BE outcome, as demonstrated by higher failure rate for Class II compounds (28%). Contrary to what is often believed, food may be a problem for Class I drugs as demonstrated by increased overall failure rate under fed conditions (14%), as compared to fasting conditions (10%). Highly variable Class II compounds have shown the highest BE failure rate (54%). The failure rate was surprisingly low (10%) for Class IV compounds. The logistic regression analysis revealed that high ISCV, fasting conditions, BCS Class II and IV were the major predictors for BE outcome. Out of all conditions analyzed, the model showed that studies conducted with low ISCV Class IV drugs, given under fed conditions, are more likely to meet BE requirements.

Conclusions: A robust and accurate model was developed which can predict and perhaps help prioritize compounds before they are moved forward into full clinical development. The findings may bring additional insights on the relationship between BCS characteristics and BE outcome and hopefully help lowering the attrition rate of new and generic product development.

This abstract was presented to the 2008 American Association of Pharmaceutical Science (AAPS) Annual Symposium and Exposition in Atlanta, Georgia, USA, November 16-20, 2008.

67. Evaluation of the Reliability Associated with Pilot Bioequivalence Studies

Jean-François Gagné, Eric Shink, Fethi Trabelsi, and Mario Tanguay. Anapharm Inc, Québec, Canada.

Purpose: Pilot studies are performed to obtain information about the test formulation and to obtain an estimate of the sample-size before conducting

pivotal studies. Anapharm's database analysis and study simulations have been performed to evaluate the risk of drawing wrong conclusions from pilot study results on the ratio point estimate (PE) and intrasubject variability (ISCV).

Methods: Retrospective analysis of Anapharm's database was done to determine study designs commonly used for the conduct of pilot bioequivalence (BE) studies. The impact of pilot study results on the outcome of the BE program was also evaluated when possible. For each of the following conditions, 1000 BE studies were simulated: theoretical PE of 85%, 95%, and 100%, ISCV between 20% and 50%, and sample-size between 12 and 36 subjects.

Results: About 50% of the 125 pilot studies evaluated used a 2-way crossover design; 69% were conducted under fasting conditions, 29% under fed conditions, and 2% under both conditions. When full pilot-pivotal programs were available (n=25), results from pilot studies allowed adequate estimation of the sample-size and resulted in good estimations of the PE (22/25 study programs resulted in at least 1 successful pivotal study). Simulations showed that given a 85% theoretical PE (bad formulation) and a sample-size of 12 subjects, the probability of detecting a bad formulation (observed PE outside 95%-105%) is $\geq 85\%$ for all ISCVs tested. However, given a theoretical ratio of 100% (good formulation), a sample-size of 12 subjects, and an ISCV of 20%, the probability of detecting a good formulation (observed PE within 95%-105%) is 48% only. This probability goes down to $\leq 30\%$ for highly variable drugs. In 12-subject pilot studies, the difference between the theoretical and the observed ISCV is $>10\%$ in 15-42% of the times for ISCV of 20-50%. Case examples supporting these results will be presented.

Conclusions: Simulation results suggest that pilot studies are efficient in discriminating bad formulations, regardless of the variability; however, for small sample-sizes, they may be associated with significant risk of drawing wrong conclusions, e.g. not detecting a good formulation.

This abstract was presented to the 2008 American Association of Pharmaceutical Science (AAPS) Annual Symposium and Exposition in Atlanta, Georgia, USA, November 16-20, 2008.

68. Subsequent Entry Biologics (SEB) for Canada: CRO Perspective of Recent EU Biosimilar Approvals (Epoetin, Somatropin, Filgrastim)

David Goblot¹, Natacha Benrimoh¹, Sophie Boudriau¹, Chloee Nadeau¹, Thomas Riel¹, Elliot Offman¹. ¹MDS Pharma Services, St-Laurent (Montreal), Quebec, Canada.

Purpose: Subsequent Entry Biologics (SEB) are biologic products that would enter the market subsequent to, and 'similar' to an approved innovator biologic. SEBs are large complex molecules manufactured in living organisms resulting in heterogeneous drug mixtures. Thus, unlike well characterized synthetic small molecule drugs, an exact "copy" of a SEB cannot be produced. This presentation will compare and contrast small molecules versus biologics, including similarities and differences in their pharmacokinetics and pharmacodynamics (PK/PD) and the clinical studies needed to assess "equivalence" or "similarity".

Methods & Results: Several SEBs or biosimilars have now been approved in the European Union (EU) using the comparability exercise approach described in the EMEA Guidelines on Similar Biological Medicinal Products. This approach included a detailed comparison of chemistry, manufacturing and controls, and numerous in vitro, non-clinical and clinical studies more akin to those needed for a new drug rather than a generic drug. For biosimilars, the comparative clinical PK studies with or without PD characterization were supportive studies with the same or wider acceptance ranges compared to small molecules. Specific clinical PK/PD studies for epoetin, somatropin, filgrastim will be presented. These PK/PD studies can be used to build confidence in a biosimilar product before the expensive pivotal comparative efficacy and safety trials.

Conclusions: The patents for dozens of biologics worth billions of dollars in worldwide yearly sales have expired or will expire in the new few years. There will be an interest to develop SEBs for Canada. The EU has taken the lead in providing regulation and legislation for biosimilars. It will be interesting to see if Health Canada will follow the same approach.

69. Clinical Program for 505 (b) (2) NDA Application – The Fast Path to Approval

Juan He, BioPharma Services Inc., Toronto, Ontario.

The 505(b)(2) application is one type of NDA, and it potentially saves pharmaceutical companies time and money. This route can be utilized for a wide range of products with limited change from an approved drug. One change suited for 505 (b)(2) application are modified release technology to achieve improved therapeutic and safety profiles with the existing approved products. There have been several approvals such as Stavzor (soft gel of valproic acid) and Zolpimist (oral spray of zolpidem).

Clinical pharmacokinetic studies comparing the bioavailability of active pharmaceutical moiety from the modified release products to that of the innovator are the important portions of the 505 (b)(2) submissions as the bridge of PK response and therapeutic efficacy. The program usually starts with the initial trial comparing overall bioavailability of the compound of interest from modified-release and innovator formulations under the fasted condition. If the desirable bioavailability is obtained, the food effect of the modified-release formulation should be tested; the consistent food effect which was seen in innovator is preferable. The steady-state comparative pharmacokinetics assessment should be carried out to investigate the accumulation of both modified-release and innovator formulations.

Drug interaction studies will provide useful information especially when the compound is metabolized via CYP 2D6 or 3A4. Chronopharmacokinetics should be assessed if the compound is known to produce different systemic and peak exposures at morning or evening dosing. The dose-strength equivalence and dose-proportionality studies are essential, if the new products will have more than one strength. The special population (renal and hepatic) PK studies are added value to 505 (b)(2) programs.

The gender and age effect should be explored in the aforementioned studies. The active metabolite(s) should be assessed for understanding of the biotransformation, and the chiral assay should be employed when the products involves the active enantiomers.

The clinical studies described above have a similar design to those within an ANDA program. The trials are 2-way crossover with 2-sequences; except for dose-proportionality and special population studies.

The trials are easily handled from clinical practice and data analysis perspectives. Such programs can provide efficient FDA approval. There are about 30 505 (b)(2) applications approved during 2008. An example of 505 (b)(2) approvals will be discussed.

70. Flexible-liposomal Gel of an Anti-inflammatory Agent: Randomized, Double-blind Clinical Trial for Evaluation of the Efficacy and Safety in Patients with Signs and Symptoms of Osteoarthritis of the Hip, Knees and Hands

Vijay Goni¹, Amit Bhatia², Sudesh Pebam¹, Tajir Tamuk¹, Asish Taneja¹, Naveen Tahasildar¹, Sakthivel Rajan¹, Basant Amarji², Bhupinder Singh², O P Katare². ¹ Department of Orthopaedic Surgery, Post Graduate Institute of Medical Education and Research, Chandigarh; ² Drug Delivery Research Group, University Institute of Pharmaceutical Sciences – UGC Centre for Advance Study, Panjab University, Chandigarh, India

Purpose: To compare epicutaneous diclofenac in flexible-liposomal gel versus marketed gel and placebo for relief of signs and symptoms in osteoarthritis (OA) of knee.

Methods: This was a randomized, double-blind, controlled trial on 30 patients with knee osteoarthritis. They were randomly assigned to flexible-liposomal formulation, active marketed formulation and placebo, three times a day for 6 weeks. The patients were assessed by primary efficacy outcome measures included the changes from baseline to end of study on the WOMAC (Western Ontario McMaster Universities) Osteoarthritis Index. The radiographic grading of OA in the knee was performed by using the Kellgren–Lawrence criteria. We also assessed the safety by evaluation of adverse events, vital signs, and irritation at the application site.

Results: In flexible-liposomal gel group the pain, stiffness and difficulty performing routine activities showed statistically significantly improvements on 6 weeks of treatment compared to the other tested formulations. All the treatments were found to be well tolerated with no adverse event.

Conclusion: Diclofenac in flexible-liposomal gel is superior to other tested formulations viz marketed gel and placebo in the relieving the symptoms of OA of the knee. Hence, it can be concluded that diclofenac in flexible-liposomal gel can be a rational alternative to oral diclofenac formulations for

management of various pain and inflammation related ailments including osteoarthritis

Clinical Sciences and Pharmacy Practice

71. Simulating the Distribution of Benefits of Catastrophic Drug Coverage in Canada

Ivana Todorovic¹, Paul Grootendorst¹, Tom Einarson¹, Audrey Laporte². ¹ Graduate Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Canada; ² Department of Health Policy, Management and Evaluation, Faculty of Medicine, University of Toronto, Canada.

Purpose: The rising importance of drugs in medical treatment and the increasing costs of drugs call for national prescription drug coverage. Although previous proposals have been modelled, the implications of the most recent one, the Catastrophic Drug Coverage (CDC) recommended by the National Pharmaceuticals Strategy (NPS), have not. This study sought to determine the impact of a nationwide CDC plan on the proportion of households that would benefit, the average gain per household and the size of overall gain.

Methods: The publicly available 2005 Survey of Household Spending contains information on out-of-pocket spending on drugs as well as the socio-economic and demographic characteristics of a representative sample of 12 million households across Canada. These data were used to estimate the distribution of CDC benefits by region of residence, household income and household type (senior, social assistance and other). Two forms of CDC were examined which determine the household deductible as a percentage of household income: Fixed (at 5%) and Variable (increasing as income increases). In some simulations, it was assumed that consumers might increase drug use in response to the availability of CDC.

Results: Analysis of the Fixed CDC option indicated 2.88% of the 12 million Canadian households would benefit with a \$426 median transfer per household, resulting in total benefit of \$299 million. The Variable CDC option generated 11.03% beneficiary households receiving a median transfer of \$282 per household, with a total benefit

of \$673 million. While both options most benefited households in Atlantic Canada and Saskatchewan, in lowest income brackets and comprised of seniors, social assistance recipients and the working poor, the Variable option particularly benefited lowest income households. When the behaviour simulations were considered, under Fixed CDC, as the average OOP expenditure increased, the additional proportion of beneficiaries would increase at a lower rate than the rate of increase in transfer amount. However, under the Variable option, for every 1 percent increase in average household OOP expenditure (across all income deciles), there would be a 0.5 percent increase in the additional percentage of beneficiaries and there would be a 3.5 percent increase in the additional overall transfer amount or “program cost” of CDC to the government, making the Variable option cost-beneficial for program sustainability.

Conclusion: The CDC proposed by the NPS benefits those in greatest need of access to affordable prescription drugs.

72. A New Chapter in the Interprofessional Collaborative Literature: Developing a Framework to Examine Professional Culture on a Family Health Team

¹Jennifer Beales, ²Murat Kristal, ^{3,1} Christine Papoushek, ¹Zubin Austin. ¹Faculty of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada; ²Schulich School of Business, York University, Toronto, Ontario, Canada; and ³Department of Family and Community Medicine, University Health Network.

Context: Governmental, educational and institutional arenas identify interprofessional collaboration (IPC) as necessary for quality patient-centred care. While family health teams (FHTs) have been created to deliver this mandate of collaborative patient-centre care, barriers such as ‘Professional Culture’ (PC) pose a challenge to attaining the interprofessional collaborative ideal. Strategies intended to advance health team collaboration inadequately examine PC, and a paucity of literature on PC further confounds our understanding of how this concept impacts IPC and the evolving FHT.

Purpose: The goal of this study is two fold: 1) to explore how PC manifests itself on the FHT and within FHT culture in an effort to develop a framework to examine PC on a FHT; and 2) examine the linkage and impact that PC has on

collaboration.

Methods: Qualitative data were collected using in-depth semi-structured focus groups (n=5). Discussions were audio-taped and transcribed verbatim. Transcripts were coded and analyzed for themes using a modified directed content analysis approach.

Participants and Setting: Non-random convenience sample of 42 participants from medicine, nursing, and allied health professions at the FHT and Diabetes Education Centre in a large academic teaching hospital in urban Canada.

Results: Analysis identifies three themes (Professional World Views; FHT World Views; and Resource Utilization) that generate a framework to depict the tension between these domains. When conflict and uncertainty are present within FHT culture, health care professionals fall back on their cognitive maps and retreat to their ‘Safe-Zones’.

Conclusions: Findings suggest that PC influences, and is influenced by FHT culture. Future ethnographic studies of FHTs would elucidate the concept of PC and be well suited to further develop this framework.

73. Tracking Medicines One Pill at a Time Using an Extension of the New BioTIFF Image Archive, Annotation, and Indexing Framework

Aria Ilyad Ahmad, West Suhanic, Jillian Kohler and Peter Pennefather. Laboratory for Collaborative Diagnostics, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario.

Purpose: Under-regulated distribution of pill form medicines, many of which have questionable provenance or are outright fakes, is a serious problem in many parts of the world. We are exploring the feasibility of developing an image based surveillance system for tracking and possibly authenticating medicines in infrastructure limited areas of the world where an ability to judge and trust information about drug quality is also limited. We are developing a technology framework for adapting widely available web-based communication strategies, as well as simple labeling, registration, and tracking protocols to deliver a flexible system for monitoring medicine quality and provenance.

Method: Principles of commodity engineering, collaborative diagnostics, and public policy analysis are being applied. We are also proving feasibility by producing cost effective robust prototype systems.

Results: A modified 64 bit TIFF image creation and annotation system called the BioTIFF is being adapted for recording and sharing images of surface features of pills and their packaging, and integrated into a scalable distributed system for using widely available edge devices to track pills at point of distribution using existing digital data communication networks.

Conclusion: Good global governance of drug quality information emerges as a key element in building trust in the value of medicines in regions where national and local governments have limited capacity to regulate drug distribution. From a technical and economic perspective, local assembly and control of medicine quality assessment infrastructure seem feasible. However, primary medication quality data needs to be explicitly linked with location-specific metadata. This linkage of data and metadata is made possible by the BioTIFF format.

Pharmaceutical and Analytical Chemistry

74. Thermal Analysis of Solutions Using Differential Scanning Calorimetry

Ermias Gebremichael¹, Kenneth Alexander⁶, Alan Riga². ¹Division of Industrial Pharmacy, Department of Pharmacy Practice, College of Pharmacy, The University of Toledo, Toledo, OH, USA; ²Department of Clinical Chemistry, Cleveland State University, OH, USA

Purpose: Both the pharmaceutical and food industry have faced fundamental challenges related to the thermal behavior of excipients, in particular sugars, which are used in the production process. The heating and cooling of sugars can produce physical and chemical transformations that can be studied using Differential Scanning Calorimetry. The main goal of this study was to evaluate the thermodynamic properties of different sugar solutions and compare them to the pure sugar samples upon heating and cooling.

Methods: The sugars investigated include Amylose, Levulose, Mannitol, β -lactose, Dextrose hydrous, and α -lactose. A fixed concentration (20% w/v) was prepared for these sugars in deionized water. Samples were placed in 100 μ l aluminum pans. The

pans were sealed hermetically and placed in a Differential Scanning Calorimetry. The temperature range was between -60 °C to 180 °C at a heating and cooling rate of 10 °C/min. The pure sugar samples ranged between 4-6 mg and were evaluated under similar conditions. All of the experiments were performed in the presence of cooled nitrogen gas.

Results: All the sugars samples showed different Differential Scanning Calorimetry curves. The sugar solutions, as well as the pure sugar samples, showed an endothermic peak related to the heat of fusion. The loss of water was observed for the aqueous sugar solutions. The exothermic phenomenon was not observed except for the mannitol solution. In all cases the endothermic peaks for the sugar solutions appeared before the pure sugar samples.

Conclusion: The thermodynamic properties of sugars upon cooling and heating vary based on the physical forms that the evaluated sugars have and the type of sugar while some of the evaluated sugar solutions have shown three endothermic peaks and most of them didn't show any exothermic peaks. The stability of pharmaceutical formulations or food products during processing or storage is in particular based on the physical and chemical properties of the excipients present in the product. This study provides fundamental information about the sugars which leads to further studies concerning the physical and chemical properties of the excipients.

75. Development of a Liquid Chromatography Tandem Mass Spectrometry Assay Method for the Determination of Proguanil, Cycloguanil and 1-(4-Chlorophenyl) biguanide in Human Plasma

Sandrine A. Mérette*, Grace van der Gugten, Hong Zhang, Winnie Liu, Rong Yi, Gina de Boer. CANTEST, Burnaby, BC, Canada.

Purpose: Proguanil is a biguanide derivative that is converted to an active metabolite called cycloguanil palmoate. It exerts its antimalarial action by inhibiting the parasitic dihydrofolate reductase enzyme, which blocks the biosynthesis of purines and pyrimidines, which are essential for DNA synthesis and cell multiplication. This leads to failure of nuclear division at the time of schizont formation in erythrocytes and liver. Proguanil has causal prophylactic and suppressive activity against *P. falciparum* and *P. vivax* and is usually given in combination with atovaquone for the treatment of malaria under the trade name of Malarone®. The

aim was to develop an LC-MS/MS method for the quantitative determination of proguanil and its metabolites, cycloguanil and 1-(4-chlorophenyl)biguanide in human plasma.

Methods: Proguanil, its metabolites and d₆-Proguanil (internal standard) were extracted from 100 µL of human plasma using protein precipitation, evaporation and reconstitution. The analytes were chromatographically separated on a Hypersil Gold AQ C₁₈ (4.6 x 50 mm) column using gradient elution with a mobile phase composed of 0.1% formic acid in 5mM ammonium formate and acetonitrile, at a flowrate of 1.0 mL/min for a total runtime of 3 minutes. LC-MS/MS was performed using Electrospray ionization (ESI) in the positive mode. Detection and quantitation were carried out by multiple reaction monitoring (MRM) scan at 254.1 to 170.1 (Proguanil), 252.12 to 195.1 (Cycloguanil), 212.00 to 60.1 (1-(4-Chlorophenyl)biguanide) and 260.1 to 170.1 (d₆-Proguanil).

Results: The method was developed over the range of 0.2 to 200 ng/mL for proguanil, 0.5 to 100 ng/mL for cycloguanil and 0.2 to 50 ng/mL for 1-(4-chlorophenyl)biguanide. Inter- and intra-batch accuracy (%RE) and precision (%CV) for standards and quality control samples are all within ± 15% (20% at LLOQ) of their nominal value for all analytes. The mean (n=4) correlation coefficient was >0.997 ± 0.0015. Matrix effects were negligible and assay recoveries were above 65% for all analytes. The stability in solution and in matrix was established.

Conclusion: A Liquid Chromatography Tandem Mass Spectrometry Assay Method for the Determination of Proguanil, Cycloguanil and 1-(4-Chlorophenyl)biguanide in Human Plasma was developed successfully.

76. Reconstituted P-Glycoprotein in Fluorosome® Lipid Bilayer Vesicles - Basis for an *in vitro* P-Glycoprotein Assay

Frances J. Sharom¹, Donald L. Melchior², George E. Wright², Ronghua Liu¹, Steven E. Wright².

¹Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON Canada;

²GLSynthesis Inc., Worcester, MA. USA.

Purpose: The need for a simple, direct method to characterize substrates and inhibitors of the P-glycoprotein multidrug transporter (Pgp, ABCB1, MDR1) led us to combine two methodologies, Fluorosome®-*trans* and functional Pgp membrane

reconstitution, to produce a prototype Pgp assay - "Fluorosome-*trans*-pgp".

Methods: Fluorosome®-*trans* is a fluorophore-containing liposome developed to measure the passive diffusion (permeability) of drugs across membrane bilayers. Fluorosome®-*trans* are unilamellar lipid vesicles (liposomes), approximately 200 nm in diameter, containing hydrophilic, drug-sensing fluorescent probes in their aqueous interior. Upon diffusion of a drug or small molecule across the bilayer, it is bound by the probe, resulting in immediate quenching of probe fluorescence. The first order dependence of the magnitude of quenching, the flux rate, is converted to the permeability coefficient of the small molecule in cm²sec⁻¹. The lipid composition of Fluorosomes can be varied to mimic the passive permeability properties of various tissues. Pgp is isolated from CH^RB30 cells by detergent (CHAPS) extraction and affinity chromatography on Con A-Sepharose. The purified micellar Pgp is mixed with micellar phospholipid and the detergent is removed by gel exclusion chromatography. The resulting lipid bilayers containing functionally reconstituted Pgp are mixed in buffer with drug-binding fluorescent probe and converted into Fluorosome-*trans*-pgp by extrusion. The resulting unilamellar vesicles, Fluorosome-*trans*-pgp, are purified by gel exclusion chromatography and have diameters of ~130 nm.

Results: Typical first order passive diffusion of test substrates into Fluorosome-*trans*-pgp is observed in the absence of the activator ATP and is identical to that in Fluorosome-*trans*. After passive diffusion reaches a steady state, addition of ATP activates those Pgp molecules with their nucleotide binding domains on the exterior of the Fluorosome-*trans*-pgp vesicle. ATP-driven inward pumping of drug molecules causes a further time-dependent increase of fluorescent quenching, reflecting active transport of the test substrate into the vesicle. Fluorescence quenching is not observed upon addition of ATP in the presence of the ATPase inhibitor sodium orthovanadate, or when the non-hydrolyzable ATP analog AMP-PNP is substituted for ATP. Reduced quenching is observed when Pgp inhibitors are added prior to ATP addition. The use of Hoechst 33342 and tetramethylrosamine as test substrates allows determination of Pgp inhibition at the putative H and R transport sites respectively.

Conclusion: The incorporation of reconstituted Pgp into the bilayers of Fluorosomes provides the basis for a rapid assay to determine if drugs are substrates or inhibitors of the transporter. This assay is

amenable to a fluorescent plate reader format.

77. CT/Optical Imaging of Lung Cancer using Liposome-based Multimodal Probes

Huang Huang¹, John Lo², Jinzi Zheng³, David Jaffray^{3,4}, Christine Allen^{1,5}. ¹Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario; ² Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario; ³Department of Medical Biophysics, University of Toronto, Toronto, Ontario; ⁴Department of Radiation Physics, Princess Margaret Hospital, Toronto, Ontario; ⁵Department of Chemistry, University of Toronto, Toronto, Ontario, Canada.

Purpose: Image guided therapy is crucial for the early detection, tracking and successful treatment of lung cancer. This study aims to develop a multimodal imaging contrast agent to effectively induce and maintain contrast enhancement in both computed tomography (CT) and fluorescence molecular tomography (FMT). This system combines high robustness of CT with high sensitivity of optical imaging, which would enable tumour tracking at the cellular level.

Methods: Cy 5.5 was conjugated to DSPE and co-

incorporated along with iohexol into liposomes (CT/optical liposomes). The physicochemical characteristics of the formulation were assessed in terms of agent loading efficiencies, size, zeta potential, *in vitro* stability, and release kinetics. The imaging properties of the liposome formulation were assessed *in vitro* using microCT and FMT in a phantom. A preliminary imaging-based evaluation of the *in vivo* stability and biodistribution of this multimodal contrast agent was also performed in healthy mice by repeated microCT and FMT imaging following intravenous administration.

Results: The purity of Cy 5.5 conjugated to DSPE was determined to be > 95%. The CT/optical liposomes showed a unimodal size distribution with a mean diameter of ~ 94nm. Quantitative analysis of CT images from the phantom study showed that Cy 5.5 does not interfere with CT contrast enhancement. This formulation demonstrated good *in vitro* and *in vivo* stability and high agent retention, comparable to predeveloped unimodal CT liposomes.

Conclusion: This study showed the feasibility of constructing a multimodal contrast agent with prolonged contrast enhancement *in vivo* for CT and optical imaging, which is useful in imaging guidance applications.

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