11th Canadian Society for Pharmaceutical Sciences (CSPS) Annual Meeting

May 22 - 25, 2008
The Banff Centre
Banff, Alberta, Canada

International Symposium on Pharmacy & Pharmaceutical Sciences:

CURRENT ISSUES IN DRUG DEVELOPMENT - EARLY 21ST CENTURY

Organizing Committee:

Laszlo Endrenyi: Co-Chair: University of Toronto, Toronto, Ontario, Canada
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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.

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Conference Program

CURRENT ISSUES IN DRUG DEVELOPMENT - EARLY 21ST CENTURY
All sessions are in Max Bell Building (MB)

Thursday, May 22, 2008	
1:00-5:30; 6:00-7:30 PM	**Registration** (1-5:30 in PDC Welcome area; 6:00-9:00 in MB Foyer)
6:00-7:30 PM	**Wine and Cheese Reception** - MB Foyer. **Exhibition** opens.

Friday, May 23, 2008	
7:00 AM - 9:00 AM 8:00 AM - 8:00 AM - 6:00 PM 8:00 AM - 6:00 PM	**Breakfast** - Vistas Dining Room **Registration** - MB Foyer **Poster Presentations** - MB 251 & MB 253 **Exhibition** - MB Foyer
	PLENARY SESSION - MB Auditorium **(Sponsored by FMC BioPolymer)** *Co-Chairs*: Rav Kumar and Elizabeth Vadas
9:00	**Introductory Remarks** by Co-Chairs
9:10	**DRUG DISCOVERY INTO THE 21ST CENTURY** Robert Young, Simon Fraser University, Burnaby, BC, Canada **(Sponsored by FMC BioPolymer)**
10:00	**Poster Viewing and NutriBreak**
10:20	**Presentations by GSK/CSPS Early Career Award Winners:** John Seubert: " Good EETs for Your Heart" Sylvia Ng: "Targeting Stromal Cells for Pancreatic Cancer Therapy"
11:30	**Luncheon - CSPS Annual General Meeting** - Donald Cameron Hall Dining Room

	Friday, May 23, 2008 (cont'd)	
1:30	**Session I a** (MB Auditorium)	**Session I b** (MB Rm. 252)
	PHARMACOGENETIC CONSIDERATIONS IN DRUG DEVELOPMENT *Organizer & Chair*: Reina Bendayan, University of Toronto, ON	**CURRENT TOPICS IN BIOANALYSIS** *Organizer & Chair*: Lorelei Lutter, CANTEST Ltd., Burnaby, BC *Co-Chair*: Xuejun Peng, CANTEST Ltd., Burnaby, BC
1:30	**Regulatory Aspects of Pharmacogenomics and Toxicogenomics** Lawrence Lesko, US Food & Drug Administration, Ellicott City, MD, USA	**Bioanalytical Issues from a Generic Industry Perspective** Keith Gallicano, Watson Pharmaceuticals, Corona, CA, USA
2:00	**Impact of Pharmacogenetics on Drug Development: An Industry Perspective** Allen Roses, Duke University, Durham, NC, USA	**A Very "Hot" Topic in Bioanalysis: Drug Biotransformation in Incurred Samples** Fabio Garofolo, Algorithme Pharma, Laval, Quebec, Canada
2:30	**Poster viewing and NutriBreak**	
3:00	**Relevance of Pharmacogenomics to Pharmacotherapy** Wayne Riggs, University of British Columbia, Vancouver, BC, Canada	**Advances in Bioanalytical Technologies for Small Molecule and Peptide Drugs** James Kapron, Thermo Fisher Scientific, Ottawa, ON, Canada
3:30	**Functional Analysis of Genetic Variants of ABC Transporters** Deanna Kroetz, University of California San Francisco, San Francisco, CA, USA	**Incurred Sample Reanalysis** Ajai Chaudhary, Lilly Research Laboratory, Indianapolis, IN, USA
4:00	**Roundtable discussion**	**Roundtable discussion**
4:30-6:00	**Poster Session Reception**- MB Foyer	

Saturday, May 24, 2008		
7:00 AM	**Breakfast**- Vistas Dining Room	
8:00 AM -	**Registration**- MB Foyer	
8:00 AM - 4:30 PM	**Poster Presentations**- MB 251&253	
8:00 AM - 3:00 PM	**Exhibition** - MB Foyer	
9:00	**Session II a** (MB Auditorium)	**Session II b** (MB Rm. 252)
	DRUG ABSORPTION AND DISTRIBUTION SESSION 1 **(Sponsored by Taiho-Alberta Foundation)** *Chair*: Glen Kwon, University of Wisconsin, Madison WI, USA *Organizer & Co-Chair*: Fakhreddin Jamali, University of Alberta, Edmonton, AB	**APPROACHES TO THE DEVELOPMENT OF DRUG CANDIDATES WITH LOW AQUEOUS SOLUBILITY** *Organizer & Chair*: Elizabeth Vadas, InSciTech Inc., Dorval, QC
9:00	**Physiological and Pathophysiological Obstacles in Drug Absorption** Fakhreddin Jamali, University of Alberta, Edmonton, AB, Canada	**How Crystal Engineering Enables the Supramolecular Design of Pharmaceutical Cocrystals with Enhanced Solubility** Nair Rodriguez-Hornedo, University of Michigan, Ann Arbor, MI, USA
9:30	**Intestinal Permeation: Prodrug Transport and Activation by Intestinal Mucosal Cells** Gordon Amidon, University of Michigan, Ann Arbor, MI, USA	**The Use of the Amorphous Form of Low Solubility Compounds to Enhance Dissolution and Oral Bioavailability** George Zografi, University of Wisconsin, Madison, WI, USA

	Saturday, May 24, 2008 (cont.)	
10:00	**Poster Viewing and NutriBreak**	
10:30	**Polymeric Micelles for Nanocombination Drug Delivery** Glen Kwon, University of Wisconsin, Madison, WI, USA	**Oral Lipid-Based Formulations: Enhancing the Bioavailability of Poorly Water-soluble Drugs** David Hauss, BristolMyersSquibb, Princeton, NJ, USA
11:00	**Pharmaceutical Applications of Polymeric and Drug Nanoparticles** Tetsuya Ozeki, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan	**Approaches of Self NanoEmulsifying Drug Delivery System (SNEDDS) & Nanosuspension for Oral and Parenteral Formulations in Cancer Therapy with Significant Impacts on Pharmacokinetics and Biodistributions** Diana Shu-Lian Chow, University of Houston, Houston, TX, USA
11:30	**Roundtable discussion**	**Roundtable discussion**
12:00-1:30	**Poster viewing and buffet lunch** in MB Exhibitor area/foyer/lounge	
1:30	**Session III a** (MB Auditorium)	**Session III b** (MB Rm. 252)
	THERAPEUTIC INHALATION PRODUCTS: ISSUES AND STRATEGIES *Organizer & Chair*: Gordon McKay, Univ. of Saskatchewan, Saskatoon, SK *Co-Chair*: Amyn Sayani, GlaxoSmithKline, Mississauga, ON	**DRUG ABSORPTION AND DISTRIBUTION SESSION 2** **(Sponsored by Taiho-Alberta Foundation)** *Organizer*: Fakhreddin Jamali, University of Alberta, Edmonton, AB *Chair*: Afsaneh Lavasanifar, University of Alberta, Edmonton, AB *Co-Chair*: Dion Brocks, University of Alberta, Edmonton, AB
1:30	**Evaluation of Bioequivalence of Locally Acting Nasal Drug Products in the US** Gur Jai Pal Singh, Watson Pharmaceuticals, Corona, CA, USA	**Molecular Biopharmaceutics: A New Era** Gordon Amidon, University of Michigan, Ann Arbor, MI, USA
2:00	**Formulation Development of Intranasal Products: Listening to the Voice of the Customer** Amyn Sayani, GlaxoSmithKline, Mississauga, ON, Canada	**Drug Targeting for Improved Distribution: Cyclosporin A** Afsaneh Lavasanifar, University of Alberta, Edmonton, AB, Canada
2:30	**Poster viewing and NutriBreak - Exhibition ends after break**	
3:00	**Methacholine Challenge: A Tale of Two Methods** Donald Cockcroft, Critical Care and Sleep Medicine, University of Saskatchewan, Saskatoon, SK, Canada	**Acrylate Nanogels and Their Microcapsular Devices for Peroral Peptide Delivery** Hideki Ichikawa, Kobe Gakuin University, Kobe, Japan
3:30	**Second-Entry Inhaled Corticosteroids - Inflammation is the Key** Irvin Mayers, Pulmonary Medicine, University of Alberta, Edmonton, AB, Canada	**Hyperlipidemia and Drug Distribution** Dion Brocks, University of Alberta, Edmonton, AB, Canada
4:00	**Roundtable discussion**	**Roundtable discussion**
6:00	**Mixer - cash bar** - Donald Cameron Hall Dining Room	
7:00	**CSPS GALA DINNER & AWARDS** - Donald Cameron Hall Dining Room **(Sponsored by Eli Lilly)**	

Sunday, May 25, 2008		
7:00 AM	**Breakfast**- Vistas Dining Room	
8:30	**Session IV a** (MB Auditorium)	**Session IV b** (MB Rm. 252)
	PERSONALIZED THERAPEUTIC STRATEGIES IN THE TREATMENT OF CANCER: FROM DISCOVERY TO IMPLEMENTATION *Organizer & Chair*: Micheline Piquette-Miller, University of Toronto, Toronto, ON; *Co-Chair*: Christine Allen, University of Toronto, Toronto, ON	**DRUG ABSORPTION AND DISTRIBUTION SESSION 3** **(Sponsored by Taiho-Alberta Foundation)** *Organizer*: Fakhreddin Jamali, University of Alberta, Edmonton, AB *Chair*: Raimar Loebenberg, University of Alberta, Edmonton, AB *Co-Chair*: Warren Finlay, University of Alberta, Edmonton, AB
8:30	**Cell-Based Assays for Targeted Discovery of Anticancer Agents** Michel Roberge, University of British Columbia, Vancouver, BC, Canada	**Advances in Respiratory Drug Delivery** Warren Finlay, University of Alberta, Edmonton, AB, Canada
9:00	**Implementation of Novel Therapeutic Strategies: Optimizing Combo-therapy of Anti-neoplastic Agents** Marcel Bally, BC Cancer Research Centre, Vancouver, BC, Canada	**Pulmonary Drug Delivery System: Inhalable Nanocomposite Particles for the Treatment of Lung Cancer** Kimiko Makino, Tokyo University of Science, Tokyo, Japan
9:30	**NutriBreak**	
10:00	**Impact of Inflammation on Drug Disposition in Cancer: Repression of Hepatic CYP3A and Drug Transporter Genes by Tumour-derived Cytokines** Graham Robertson, ANZAC Research Institute, Sydney, NSW, Australia	**The Biopharmaceutics Drug Classification System and Quality by Design** Raimar Loebenberg, University of Alberta, Edmonton, AB, Canada
10:30	**Novel Drug Delivery Strategies: Localized and Sustained Intraperitoneal Delivery of Taxanes for the Treatment of Ovarian Cancer** Christine Allen, University of Toronto, Toronto, ON, Canada	**Updated Guidance for the Conduct and Analysis of Bioequivalence Studies** Eric Ormsby, Health Canada, Ottawa, ON, Canada
11:00	**Roundtable discussion**	**Roundtable discussion**
11:25	**Concluding Remarks**: Elizabeth Vadas	**Concluding Remarks**: Laszlo Endrenyi
11:30	**Conference Adjourns**	

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SYMPOSIUM 2008 AWARDS

CSPS Award of Leadership in Canadian Pharmaceutical Sciences:

Recipient: Robert Young, Ph.D., FRSC, OC, Merck Frosst-BC Discovery Chair in Pharmaceutical Genomics, Bioinformatics and Drug Discovery, Department of Chemistry, Simon Fraser University, Burnaby, British Columbia

CSPS/GlaxoSmithKline Early Career Award:

Co-Recipient: Sylvia Ng, Ph.D., Senior Scientist, Department of Advanced Therapeutics at the British Columbia Cancer Agency; Assistant Professor in the Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia

Co-Recipient: John Seubert, Ph.D., Assistant Professor, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB

Gattefosse Canada/CSPS Lipid-Based Drug Delivery Award:

Recipient: Kaley D. Wilson, *et al.* (Laboratory of Dr. Pieter Cullis, University of British Columbia) for their manuscript entitled "*Effects of intravenous and subcutaneous administration on the pharmacokinetics, biodistribution, cellular uptake and immunostimulatory activity of CpG ODN encapsulated in liposomal nanoparticles*" published in International Immunopharmacology 7 (2007) 1064–1075.

Poster Awards - Winners to be announced at 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.:

Name of Recipient	Supervisor(s)	Title of Project	University/ Administrator
Leslie Leung	Dr. Ujendra Kumar	Regulation of the membrane expression of somatostatin receptor subtypes (SSTR 1 to 5) under tamoxifen and estradiol treatment and the effect of oxidative stress on the cell-death pathway in human breast cancer cells	University of British Columbia
Christine Leong	Dr. Mike Namaka	Nasal Colonization of *Staphylaococcus aureus* in Relapsing Remitting Multiple Sclerosis Patients	University of Manitoba
Travis Featherstone	Dr. Scot Simpson	Characterizing Use of Acetylsalicylic Acid Within a Long Term Nursing Home Facility	University of Alberta
Jason Wentzell	Dr. David Jakeman	Cloning and characterization of the metabolic genes involved in the biosynthesis of L-digitoxose	Dalhousie University
Alexandre Meunier	Dr. Stephane Angers	Characterization of FLJ10324:a novel protein connecting heterotrimeric and small G proteins	University of Toronto

Speaker Abstracts

Plenary Sessions

Drug Discovery into the 21st Century

Robert N. Young, Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada

Methods and approaches to drug discovery have evolved rapidly over the last decade or so, fuelled by the biotechnology and genetics revolution. At the same time the public demand for safer and more cost and medically effective drugs has continued to raise the bar for success in drug discovery and development. Advances in understanding of human genetics have provided a myriad of potential drug targets but determining which of these will yield useful drugs is a challenge. Human genetic profiling can identify genes associated with disease and methods such as gene knock-out, antisense and siRNA can provide strong evidence for relevance of such targets. Small molecule probes and compounds with unusual properties can also be employed as tools of "reverse pharmacology" to uncover novel drug targets. Our improved understanding of human physiology has also allowed rapid advances in the design and optimization of new drugs to provide molecules with predictable human half lives and controlled routes of metabolism and elimination. Once a day drugs are now the norm and once a week and even once a year drugs are now being brought into use. We are now able to mitigate potentials for drug-drug interactions, off-target toxicities and side effects to provide superior treatments with the physical, chemical and biological properties needed for inclusion in the 21st century pharmacopeia. Several recent examples of target discovery and drug optimization will be described.

Good EETs for Your Heart

John M. Seubert, Faculty of Pharmacy, University of Alberta, Edmonton, AB, Canada

As the population ages and co-morbidities such as obesity and diabetes become more prevalent; both the human cost and economic burden of cardiovascular diseases (CVD) will likely increase. Acute myocardial infarction (AMI) continues to be a leading cause of death worldwide. Myocardial infarction occurs when ischemia exceeds a critical threshold and overwhelms cellular repair mechanisms resulting in irreversible myocardial cell damage or death. This is the primary factor in the pathogenesis of ischemic/reperfusion injury and myocyte loss associated with CVD. Advances in early reperfusion therapy, such as thromobolytic drugs, coronary angioplasty or bypass graft surgery, have reduced morbidity, heart failure and infarct-associated ventricular arrhythmias. Unfortunately, both early reperfusion therapy and cardioprotective drugs given prior to ischemia have limited clinical utility as patients typically present after the onset of ischemia and/or are unable to reach medical facilities. Targeting strategies that limit the damage has been a major focus of research. Despite substantial research in cardioprotective mechanisms, the increasing incidence and prevalence of heart failure after AMI highlight the need for a better understanding of the pathophysiological mechanisms. Arachidonic acid (AA), a polyunsaturated fatty acid normally found esterified to cell membrane glycerophospholipids, can be released by phospholipases in response to several stimuli such as ischemia. Cytochrome P450 epoxygenases metabolize AA to epoxyeicosatrienoic acids (EETs) which are converted to dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (Ephx2, sEH). CYP-derived metabolites of arachidonic acid play critical roles in modulating fundamental biologic processes. Environmental or genetic factors that alter P450 or sEH expression and/or function lead to changes in production of bioactive eicosanoids. Such effects can influence cell and organ function in either an adverse or beneficial manner. Traditionally, investigation into the role of CYP isozymes has generally focused on hepatic and renal drug metabolism and function. There is little known about the importance of this endogenous system within the heart. Interestingly, recent human epidemiological evidence has identified a correlation between CYP2J2 polymorphisms and coronary artery disease, as well as EPHX2 and coronary heart disease. Targeting the cytochrome P450 metabolites of arachidonic acid, either through pharmacological or gene therapy mechanisms, represents a novel therapeutic approach to management of ischemic heart disease in humans. Ultimately, understanding the basic cellular mechanisms of EETs will enhance our knowledge and lead to better management of cardiovascular diseases.

Targeting Stromal Cells for Pancreatic Cancer Therapy

Sylvia Ng. Department of Advanced Therapeutics, BC Cancer Agency & University of British Columbia, Vancouver, BC, Canada

Pancreatic adenocarcinoma remains a therapeutic challenge in oncology to date. It has the worst survival rates of any cancer - with a typical life expectancy of 3−6 months after diagnosis. This cancer is prone to early metastasis and is extremely resistant to chemotherapy and chemo-radiotherapy. Much research effort has been devoted to enhancing chemosensitivity through the inhibition of cancer cell survival pathways with novel molecularly targeted agents. However, tumor initiation and progression are now recognized to be the product of continuous communication between tumor cells and their surrounding supporting tissues, collectively known as tumor stroma. The predominant cell type in the tumor stroma is cancer-associated fibroblasts (CAFs), which have been shown to induce tumor growth and promote tumor progression. We speculate that targeting cancer cells alone is not sufficient to improve response to chemotherapy, especially in pancreatic cancer which is characterized by a strong desmoplastic reaction in association with extensive fibroblast proliferation and modified extracellular matrix (ECM) deposition. Our hypothesis is that CAFs, being the dominant cell type and the principal producer of growth factors and ECM proteins in the pancreatic tumor stroma, may significantly influence the tumor's response to chemotherapy. The main objective of our research is to identify proteins in pancreatic CAFs that are critically involved in mediating communication with endothelial cells or cancer cells, and in modifying response to chemotherapy. Significance: The identified proteins have great potential to act as new therapeutic targets for the development of new drugs in the treatment of this devastating malignancy.

Session I

a. Pharmacogenetic Considerations in Drug Development

b. Current Topics in Bioanalysis

SESSION Ia

Regulatory Aspects Of Pharmacogenomics And Toxicogenomics

Lawrence Lesko, US Food & Drug Administration, Ellicott City, MD, USA

The regulatory framework around pharmacogenomics (PGx) and toxicogenomics (TGx) can have a significant effect on the adoption of these sciences in drug development and clinical practice. Therefore, beginning in 2002, the FDA made a conscious decision to proactively advance the field of PGx and TGx by committing time, people and technology to enable the science. It began by co-sponsoring a large number of public workshops to define the current status and future direction of the field. These workshops led to the "Guidance on Pharmacogenomic Data Submission" in 2005 which defined when PGx and TGx data needed to be submitted to FDA and when the data could be submitted voluntarily. The FDA established a voluntary genomic data submission (VGDS) process and an Interdisciplinary PGx Review Group (IPRG) to review these submissions and meet with sponsors. To date, FDA has received over 40 VGDS packages. In 2007 and 2008 VGDS meetings were initiated with regulatory colleagues from Europe and Japan reflecting the global nature of drug development. The FDA was also a major player in the development of the first ICH guideline related to PGx focusing on definitions and biospecimen sample coding (E15). A spin-off from the VGDS process was the Biomarker Qualification Process (BQP) which FDA established in a coordinated fashion with the EMEA and JPMA regulatory authorities. Recently a new panel of 6 preclinical safety biomarkers for predicting drug-induced renal damage was qualified via this process using data that originated from a consortium of industry partners. Facilitating the development of targeted therapies in new drug development has been a focus of the FDA genomics strategy with approvals such as Selzentry^{R} (maraviroc) for the treatment of HIV-AIDS when coupled with a companion diagnostic for identifying tropism in the virus. The Agency has also looked at previously approved drugs to determine if adding genetic tests to the approved labels has the potential to improve the benefit/risk ratio. Thus, labels of 6-mercaptopurine, irinotecan, carbamazepine and warfarin have been updated with PGx information. Next steps in the evolution of regulatory PGx and TGx is do develop a new guidance for industry on "clinical pharmacogenomics" which will address DNA collection during drug development and the types of PGx studies that might be of most value. The FDA is also looking at safety PGx for previously approved drugs and new drugs and working through public-private partnerships to identify predictive genomic biomarkers.

Impact of Pharmacogenetics on Drug Development: An Industry Perspective

Allen D. Roses, Deane Drug Discovery Institute, Duke University Medical Center and Fuqua School of Business, and Cabernet Pharmaceuticals, Inc., Durham, NC, USA

From a pharmaceutical R&D viewpoint the most critical step in the development of a drug is the proof of concept [efficacy]. Generally in large pharmaceutical companies, there is a surplus of new candidate leads that have survived animal safety testing. Many points of view within a company may decide which drug candidates [DCs] are carried to First Time In Human and Phase 1 and II human testing. Frequently these decisions are influenced strongly by commercial market decisions that are based on the belief that the DCs use is limited to a specific disease or therapeutic area. When pathways are well established, as several are in oncology, the choice of therapeutic area is clearer than for drug designed for CNS or metabolic disorders. The "best" place to suffer attrition of a DC asset is at Phase II POC, before the relatively enormous costs of Phase III and Phase IV clinical trial programs. Unfortunately this is the step in the pipeline when clear clinical efficacy end-points are the most difficult to demonstrate with statistical significance using the Intention-to-Treat population [ITT, "all comers"]. However, if only 20%-50% of a patient population demonstrate objective efficacy in response to the drug, than averaging the therapeutic effect across the whole population loses the efficacy signal. Examples will be demonstrated where pharmacogenetic analyses during Phase II POC testing yielded clear efficacy signals, following failed standard ITT analyses that were classic for attrition. If the expected population with efficacy can be projected in subsequent clinical trials, the identified genetic markers may define a robust prognostic test for drug therapy that can be sharpened [clear false positive signals in test panel] with subsequent clinical trials. Safety PGX and pharmacovigilance are critical to the longevity of the developmental program as well as maintaining and protecting the market place after drug registration. Some safety signals can be tolerated, especially when the drug is

meant to treat life-threatening severe diseases [like HIV or cancer]. The most expensive potential adverse events {AEs} are those that occur once a drug is in the market-place with the full cost of R&D, launch and marketing. Even more disturbing to industry is the trend to impeach drugs on potential statistical support for the possibility of an adverse event. These are particularly note-worthy and developers spend considerable resources on Phase IV clinical trials to create real data. If AE-based PGX analyses of actual events can be identified by the development of a prognostic or diagnostic test, the standard of evaluation would shift from statistics and politics [including academic advancement] to accurate data on patients' actual responses to drugs. Multiple examples will be demonstrated that speak to "How few patients does it take" to recognize an AE and rapidly determine genetic tests to identify those specific patients who should not take a particular drug. True individualized medicine increases the chance of drug efficacy for individuals and, in well studied cases, can predict the susceptibility for adverse events for an individual with surprising accuracy [high sensitivity and specificity].

Relevance of Pharmacogenomics to Pharmacotherapy

K. Wayne Riggs, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada

Pharmacogenomics utilizes a multiple gene or genome-wide approach to identify genetic determinants of drug efficacy and toxicity as a consequence of genetic variations (single nucleotide polymorphisms; SNPs) in drug targets, transporters or drug metabolizing enzymes. Ideally this information will provide the opportunity for personalized healthcare – the ability to consistently deliver the right drug treatment to the right patient at the right time thereby improving drug effectiveness, reducing adverse effects and providing cost-effective pharmaceutical care. How close are we to this reality? It has been known for several decades that polymorphisms in a single gene coding for drug metabolizing enzymes can have important implications for drug dosing. In spite of this, the routine application of pharmacogenomics in clinical drug treatment is still at an early stage and likely will be for some time given the current model of healthcare. There are, however, incremental successes with some 20 different cases where the FDA now requires, recommends or provides information for genotyping. Examples include warfarin (CYP2C9, VKOR), Herceptin (HER2 receptor overexpression), Gleevec (gastrointestinal stromal tumors), maraviroc (HIV-CCR5 receptor site), celecoxib (CYP2C9), cetuximab (EGFR receptor), azathioprine (TPMT deficiency) and irinotecan (UGT1A1; homozygous for the *28 allele). While we may dream of a genetic biomarker for each and every drug this certainly will not be a reality in the near future. Rather, pharmacogenomic applications will be most relevant for drugs which have a narrow therapeutic index and high variability in response, for drugs in which response is difficult to measure, and for the molecular diagnosis of disease (e.g., oncology, infectious diseases, respiratory disease, cardiovascular, mental health). Within the next decade technology will most likely allow us to achieve the promise of the $1000 genome for an individual, but are we as health care providers, the public and regulatory bodies ready to routinely apply this information in pharmacotherapy? This will mean a change in the blockbuster drug philosophy of 'one-size-fits-all', the re-education of physicians from decades of training to start treatment with the default 'average dose' and the 'trial and error' approach which is presently widely accepted. Pharmacogenomics – hype or reality? Only time will tell.

Functional Analysis of Genetic Variants of ABC Transporters

Deanna L. Kroetz, Department of Biopharmaceutical Sciences, University of California San Francisco, San Francisco, CA, USA

The ATP-Binding Cassette (ABC) superfamily of membrane transporters is involved in the efflux of a wide range of xenobiotic and endogenous substrates. The high expression of ABC transporters in eliminating organs contributes to the pharmacokinetic properties of drugs. ABC transporters are also expressed at barrier sites (e.g., the blood-brain barrier and the maternal-fetal barrier) and in peripheral tissues where they limit cellular exposure to xenobiotics. Understanding the mechanisms of interindividual variation in ABC efflux transport function is critical for optimizing drug therapy. Genetic polymorphisms in ABC transporters are one potential source of interindividual variation in transport function. As part of a large, multi-investigator project on the pharmacogenetics of membrane transporters, we have resequenced the coding and proximal promoter regions of the major ABCB, ABCC and ABCG transporters involved in drug efflux. Deep resequencing in ethnically diverse populations has identified significant genetic diversity in these transporter genes. In general, genetic diversity is

greater at synonymous sites than non-synonymous sites in the coding region, suggesting some degree of selective pressure to limit changes in transporter function. Surprisingly, for some transporters, genetic variation is greater in the proximal promoter region than in the coding region. A major hurdle in advancing the large amounts of data on genetic variation into the clinic is the lack of studies addressing the functional impact of genetic polymorphisms in these transporters. Using heterologous expression systems, we have studied the impact of nonsynonymous polymorphisms on transporter function. Computational studies have been used to predict those polymorphisms most likely to alter function. Several P-glycoprotein and MRP4 variants show altered transport of clinically relevant substrates. In many cases, these differences are substrate-dependent, which has significance in the design and interpretation of clinical association studies. Polymorphisms in the UTRs and the promoters of the ABC transporters are also being investigated in in vitro systems. Following the identification of functionally relevant genetic polymorphisms, genotype-to-phenotype studies can be carried out to examine the clinical significance of changes in transporter function and/or expression. The ultimate goal of these studies is to translate these findings into clinical approaches to enhance the efficacy and reduce the toxicity of widely used drugs.

SESSION Ib

Bioanalytical Issues from a Generic Industry Perspective

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

To meet today's generic regulatory requirements, labs are challenged with many difficult and highly sensitive assays for parent drug that were not available when the innovator filed its application. Thus, the reliability of the assay becomes critical for a successful generic application. Sponsors want assurance that the assay is reliable for incurred samples, not just for spiked samples. With the increased use of liquid chromatographic coupled with mass spectrometric detection methods (LC-MS), more problems with incurred sample accuracy are occurring than with the older, less used HPLC assays, whereas incurred sample reproducibility was more problematic with HPLC assays in the early 1990's. Incurred sample accuracy issues are related to problems with assay selectivity (metabolite interference or interference in blank matrix) and sample processing (matrix effects and metabolite conversion to parent drug). Potential accuracy issues must be addressed during method development and validation, preferably by cross-validation with another assay using incurred samples, because an inaccurate assay can still be reproducible. This presentation will highlight examples of how problems related to assay selectivity were discovered either before and after ANDA submissions to FDA, how the Sponsor and CROs worked to together to solve the problems, and what impact the assay issues had on the ANDA submission.

A Very "Hot" Topic in Bioanalysis: Drug Biotransformation in Incurred Samples

Fabio Garofolo*, Melanie Bergeron, Jean-Nicholas Mess and Milton Furtado, Algorithme Pharma Inc., Laval (Montreal), QC, Canada

Acyl glucuronidation is an important metabolic pathway for drugs with a carboxylic acid moiety. These metabolites are found to be labile and may revert back to the parent drug under particular physiological conditions. Lack of literature on the stability of specific acyl glucuronides and pharmacokinetic data (Cmax, Tmax) truly hinder the course of method development. It is therefore essential to determine the impact of the conjugated metabolite on the quantitation of the parent drug at each step of method development. After an extensive literature research, acyl glucuronides seem to be more stable in acidic conditions but some results show otherwise. Even if acidic conditions are chosen, the acyl glucuronide stability could impact on drug quantitation and should be routinely tested. All acyl glucuronides tested have shown to be stable in fresh whole blood at 4°C for a period of 2 hours. In plasma samples kept at 4°C without preservative, the Clopidogrel acid glucuronide is stable for at least 48 hours. The addition of acidic preservative, which is supposed to stabilize the acyl glucuronide according to the literature, promoted the hydrolysis of the glucuronide to the parent drug. However, Etodolac acyl glucuronide showed to be slightly unstable in plasma at room temperature while degradation was inhibited by the addition of acidic preservatives in the plasma. Therefore, acyl glucuronide stability in plasma samples cannot be predicted. Freeze-thaw cycles do not affect conjugated metabolite stability in plasma with and without preservative for the 3 compounds tested. No

significant conversion of the acyl glucuronide of Etodolac and Repaglinide was observed during the liquid-liquid-extraction (LLE) process even when an extreme pH (1.2N HCl) is used. The autosampler stability of Clopidogrel Acid in presence of the acyl glucuronide at pH=3.0 for 96 hours at 4ºC had a % deviation of 446.1%, however at pH=7.0; the % deviation is 3.2%. It is therefore imperative to evaluate the autosampler stability for drugs in the presence of their conjugated metabolites to avoid discrepancy during incurred sample analysis. For Etodolac and Repaglinide, since the extraction efficiency of the glucuronide in LLE is very low, the autosampler stability of the glucuronide has little impact on the quantitation of the parent drug. In conclusion, the stability of acyl glucuronides cannot be generalized since the aglycone has an impact on the stability of the glucuronide. An extensive stability evaluation should be routinely performed in the bioanalytical laboratory when acyl glucuronides exist as major metabolites in plasma.

Advances in Bioanalytical Technologies for Small Molecule and Peptide Drugs

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The pharmaceutical industry wants value for money (cheaper), but researchers are still keen to solve their problems quickly (faster) and elegantly (better). This presentation will demonstrate two technologies developed right here in Canada and being used world-wide to more efficiently discover new pharmaceutical remedies. The first technology is Laser Diode Thermal Desorption (LDTD). Invented in Québec, this technology uses a laser to volatilize biological samples before ionization. LDTD shares similarities with APCI and MALDI but possesses key technological differences including: no ionization matrix required, atmospheric pressure ionization and laser energy in the infrared range instead of ultraviolet. Features of LDTD include low sampling volumes (as low as 3uL), no sample pre-treatment and no sample-to-sample carryover. For a representative analysis of midazolam in plasma, the accuracy and precision of the assay were 13% difference and 11% RSD, respectively. The benefit of LDTD is rapid analysis of sample-volume challenged quantitative assays (7 seconds per sample). The second technology is high-Field Asymmetric waveform Ion Mobility Spectrometry (FAIMS). The latest developments in the use of this technology is in the GLP arena. FAIMS is used to remove interferences in small molecule and peptide quantification methods. A quantitative assay without FAIMS was limited by matrix interferences at the lower level of quantitation (LLOQ) because matrix blanks contained up to 35% of the LLOQ signal. By introducing FAIMS, the accuracy and precision of the assay were unchanged (accuracy 8% difference, precision 10% RSD), but the interferences in the confirmation of LLOQ experiment were reduced below the generally accepted level of <20% of the LLOQ response. The method was subsequently validated for the analysis of rat plasma samples. The benefit that FAIMS offers is selectivity by reducing interferences.

Incurred Sample Reanalysis

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AAPS Workshop on "Current Topics in GLP Bioanalysis: Assay Reproducibility for Incurred Samples—Implications of Crystal City Recommendations" was held on February 7-8, 2008, Arlington, VA. The "Crystal City III" Conference Report left it up to the bioanalytical community to discuss not <u>if</u> incurred sample reanalysis (ISR) is needed but <u>how</u> to do it. This workshop allowed a forum for discussion and sharing ideas on how best to do ISR. The recommendations from this workshop will be presented.

Session II

a. **Drug Absorption and distribution (Session 1)**

b. **Approaches to the development of Drug Candidates with Low Aqueous Solubility**

SESSION IIa

Physiological and Pathophysiological Obstacles in Drug Absorption

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Following oral dosing, rapid and efficient drug absorption is often desirable. Slow and/or erratic absorption are often attributed to factors such as low aqueous solubility and inefficient transport systems. Hence, attempts are usually made to overcome the absorption problems by enhancing solubility or rendering the transfer system more efficient. In general, however, the 'proof of concept' is tested in humans and/or in animals models under healthy conditions assuming that improved absorption translates to improved onset of action or effectiveness. However, evidence have been emerging suggestive of a lack of parity between the healthy and disease conditions with regard to the absorption patterns, hence, improved therapeutic outcomes. Indeed, a rapid absorption in healthy volunteers of readily soluble formulations does not necessarily translate to improved onset or efficacy; e.g., ibuprofen soft gelatin capsules, anti-migraine triptan formulations. Despite substantially faster drug absorption in healthy volunteers, slow absorption has been observed in patient in pain. This is due to the slow disintegration and dissolution during pain episode secondary to suppression of the vagus nerve mediated by pain or its trauma. Other conditions that may alter drug bioavailability include Parkinson's disease, arthritis, Behçet's disease, renal impairment, celiac and other gastrointestinal diseases, old age, gluten intolerance. In addition, altered presence of proteins involved in the transport of drugs. For example, at least in vitro and in vivo in rodents, inflammatory conditions result in reduced expression of several transporters such as p-glycoprotein and various multiple drug resistance proteins. Altered expression of these proteins may influence drug bioavailability although human data yet to be reported. Attempts to improve physicochemical properties of pharmaceutical products may fail if pathophysiology of the patient is ignored.

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Intestinal Permeation: Prodrug Transport and Activation by Intestinal Mucosal Cells

Gordon L. Amidon, College of Pharmacy, University of Michigan, Ann Arbor, Michigan, USA

Our understanding of the membrane transport mechanisms of drugs has undergone a revolution in the past 10 years. Epithelial cells, in particular, lining the gut, liver, and kidney represent the principle barrier for drug entry and exit from the body and they are an essential determinant of the ADME properties of drugs. A more recent discovery was that the membrane transport pathway of prodrugs was shown to be carrier mediated. Today it is firmly established that several peptidomimetic prodrugs utilize the peptide carriers, hPEPT1 for entry into the mucosal cell. In particular the demonstration that nucleoside prodrugs utilize a carrier mediated pathway for mucosal cell entry, explains the high bioavailability of valacyclovir. However, a second step is required for a prodrug to be effective, that of activation. This step is often relegated to ubiquitous 'esterases'. Recently we identified a novel esterase enzyme, Valacyclovirase, (also known as BPHL) and have shown VACVase to efficiently hydrolyze the esters of valacyclovir and valganciclovir. This presentation will provide an over view of membrane transport of nucleoside prodrugs, focusing on epithelial cell transporters and reports the recent results of our studies on the activation, by Vacvase, of a variety of nucleoside prodrugs. We conclude that, with the tools available today, prodrug design can be based on detailed molecular mechanisms and strategies.

Polymeric Micelles for Nanocombination Drug Delivery

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Amphiphilic block copolymers assemble into micelles–nanoscopic supramolecular core/ shell structures, which are increasingly being researched for drug delivery. The design and chemistry of polymeric micelles is now relatively diverse, but must consider drug loading (usually drug solubilization), safety for IV administration, circulation in blood, and drug release; extracellular or intracellular drug release in a sustained, controlled, or triggered manner. We have extensive experience in the synthesis of amphiphilic block copolymers for drug delivery and have widely researched polymeric micelles for drug solubilization. We have studied simple physical methods for drug solubilization as well as chemical prodrug strategies that rely on hydrolysis for drug release. In the former case, we have shown that simple changes in the side chains of the core-forming block can be used to tune drug release. In the latter case, drug release can be dependent on pH, providing a strategy for triggering drug release in the endosome/lysosomal pathway. We continually research polymeric micelles for drug solubilization as a first step in drug administration and drug action, as an alternative to cosolvents and surfactants used commonly for pre-clinical drug development. We believe that polymeric micelles will be an integral component of drug delivery, particularly in efforts for drug targeting that aim to utilize their small size ("small ball") for innovative combination therapies, which may involve two or more drugs.

Pharmaceutical Application of Polymeric and Drug Nanoparticles

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The 4-fluid nozzle spray drier has a unique nozzle with two liquid and two gas passages, which allows drug and carrier to be dissolved in separate solvents, thereby avoiding the need for a common solvent. The composite microspheres containing nanoparticles of polymers and water-insoluble drugs were prepared using the spray drier. Size control of polymeric nanoparticles dispersed in microspheres and the production mechanism, and enhancement of oral and pulmonary absorption of the water-insoluble drugs were investigated. We developed a novel original spray nozzle which makes polymer or drug to be nano-sized particles dispersing in microspheres using an anti-solvent effect. Biodegradable poly(lactic-glycolic acid) (PLGA) and ethylcellulose (EC) were used as polymers. Pranlukast hydrate (PLH) was used as a water-insoluble drug. Rifampicin (RFP) was used as an anti-tuberculosis drug. Mannitol (MAN) was used as a carrier of microspheres. The acetone/methanol or ethanol/sodium carbonate solutions of polymers or PLH and the aqueous solution of MAN were simultaneously supplied through different passages of the 4-fluid nozzle or the original nozzle, and the spray dried to obtain the composite microspheres containing PLGA, EC or PLH nanoparticles. Polyethylenimine (PEI) was added to load cationic charge to PLGA. The PLGA nanospheres with about 200 nm in diameter could be prepared. The zeta-potential of PLGA-PEI nanospheres showed positive, suggesting the possibility of carrier for gene delivery by absorbing DNA on the surface. The oral absorption of PLH from PLH/MAN composite microspheres in rats was improved compared to that of PLH powder and the prolonged absorption was observed. The pulmonary absorption was markedly high and almost 100-fold of AUC compared to the oral administration of PLH powder was observed. We also developed the particles fro inhalation therapy of tuberculosis and studied the delivery to the lung and uptake to the alveolar microphages in rats. The EC particles which are approximately 100 nm in size were prepared in one-step by using an original spray nozzle.

SESSION IIb

How Crystal Engineering Enables the Supramolecular Design of Pharmaceutical Cocrystals with Enhanced Solubility

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The emerging field of crystal engineering enables the generation of families of cocrystals of a drug with different molecular components. The ability to design and build hierarchical structures from molecular or supramolecular modules is based on noncovalent interactions, and often hydrogen bonds between neutral molecules. This means that cocrystal formation offers unique opportunities to enhance or modify properties inherent to a drug (solubility, dissolution, bioavailability, stability, etc.) depending on the nature of the second component or coformer. Strategies for cocrystal design rely on developing a supramolecular library of coformers, based on crystal packing and hydrogen bond functionalities. The Cambridge Structural Database can be analyzed to identify robust molecular motifs among crystal structures with molecular components of interest. Cocrystals are screened and synthesized by solution, solid-state or melt processes. Mechanisms for cocrystal formation are emerging and involve supersaturation- or amorphous-mediated processes. Recent findings show that for some drugs as many as 40 to 50 cocrystals can be generated. Although solubility, dissolution and bioavailability studies are scarce, a few reports suggest that cocrystals are often more soluble and bioavailable than parent drug. This presentation will describe the theoretical aspects behind cocrystal design and formation by solution and solid-state processes, and the importance of phase diagrams to develop cocrystal screening methods and to anticipate transformations during processing and storage. Modification of solubility, dissolution and bioavailability through the formation of cocrystals will also be discussed.

The Use of the Amorphous Form of Low Solubility Compounds to Enhance Dissolution and Oral Bioavailability

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In producing a potentially active pharmaceutical ingredient (API), process chemists prefer to isolate the solid as crystals since the crystalline state provides high levels of purity and generally provides maximal chemical and physical stability. Over the past few years, however, it has become increasingly apparent that as crystals, a higher proportion of potential drug molecules are exhibiting very low solubility in water, leading to poor dissolution rates and correspondingly low oral bioavailability. In part, this is due to a trend toward discovering active molecules with larger molecular weights than previously observed. Besides often leading to more hydrophobic molecules, such increased molecular weight also can lead to stronger crystal lattice energies, as reflected in higher melting temperatures and corresponding decreases in solubility. Consequently, despite the presence of hydrogen bonding groups that might exhibit a strong affinity for water and good aqueous solubility, this often also leads to stronger intermolecular interactions within the crystal and stronger lattice energies that must be overcome to produce dissolution. To counter this, crystalline solids can be rendered non-crystalline or amorphous so that a solid lacking these strong lattice energies can be formed with significantly greater solubility than the crystalline counterpart. In this talk we will examine the characteristics of molecules in the amorphous state that must be considered in using the amorphous state to enhance dissolution and oral bioavailability. Recognizing that molecules in the amorphous state are thermodynamically unstable relative to those in the crystalline state, and therefore, capable of recrystallizing during the period of product storage, as well as at the time of release in the g.i. tract, we will discuss various factors that can influence the crystallization of molecules in the amorphous state, including temperature, relative humidity and the presence of various excipients. In particular we will focus on the use of water-soluble polymers to form amorphous molecular dispersions that can inhibit the tendencies for crystallization during the desired shelf life of the product and after administration.

Oral Lipid-based Formulations: Enhancing the Bioavailability of Poorly Water-Soluble Drugs

David J. Hauss, Development Metabolism and Pharmacokinetics, Bristol-Myers Squibb Company, Princeton, NJ, USA

Poor drug solubility remains a significant and frequently encountered problem for pharmaceutical scientists. The ability of lipid-based formulations to facilitate gastrointestinal absorption of many poorly water-soluble drug candidates has been thoroughly documented in the published literature. However, a considerable gap exists between our knowledge of this technology and the know-how required for its application. This presentation will provide an overview of the need for, and application of, oral lipid based formulations. Biopharmaceutical aspects of drug absorption from lipid matrices will be reviewed along with drug physiochemical determinants for successful application of these formulations. A forward-looking summary of unrealized opportunities, potential limitations, and challenges to more widespread use of this technology will be provided.

Approaches of Self NanoEmulsifying Drug Delivery System (SNEDDS) and Nanosuspension for Oral and Parenteral Formulations in Cancer Therapy with Significant Impacts on Pharmacokinetics and Biodistributions

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Nanoemulsifying drug delivery system (SNEDDS) and nanosuspension have been employed as strategies to formulate highly lipophilic anti-neoplastic agent, mebendazole (Mbz), of aqueous solubility of 0.7 μg/ml, for oral and parenteral administrations. The SNEDDS, pre-nanoemulsion concentrates (without the aqueous phase), are isotropic mixtures of oil, surfactant/cosurfactant, cosolvent/solubilizer and the drug with the droplet size in the range of < 50 nm – 500 nm. The SNEDDS are classified into Types I, II, IIIa and IIIb, depending on the compositions. For SNEDDS, systematic approaches in selection of excipients and identifying the nanoemulsion regions using ternary phase diagram enable the effective formulation optimizations. The SNEDDS-based nanoemulsions of 35 nm and 143 nm significantly improved the oral bioavailability of Mbz, 228 and 120 times of that from unformulated suspension, respectively, in SD rat model. With parenteral administrations SNEDDS-based nanoemulsions, Mbz yielded prolonged half-life, 644 min vs 173 min from the cosolvent reference (CS), and sustained exposures in organs, especially in the lung. Significant impacts of droplet sizes (35 and 478 nm) of SNEDDS-based nanoemulsions on biodistributions of Mbz were demonstrated with an even higher concentration and a longer retention half-life with the smaller sized formulation, in both SD rat and athymic mouse models. For nanosuspensions, the formulation theory, selections of the type and amount of stabilizer, and techniques of nanoparticle preparation will be discussed. Four size ranges of Mbz nanosuspensions, 128-167 nm, 250-253 nm, 739-891 nm and 1552-1781 nm, were prepared using milling technique. The Mbz from nanosuspensions exhibited prolonged half-lives, 13-30 hr vs 3 hr from CS. The Vss were 1.19-1.69 L vs 0.06 L from CS in the mouse model, and peripheral V_2 were substantially larger, 24-49 L vs 0.4 L from CS in the rat model. The size impacts were demonstrated. Greater tissue distributions and slower in vivo dissolution were key parameters responsible for the size-dependent distinction of Mbz pharmacokinetics. Both SNEDDS and nanosuspension offer potential merits of sustained and targeted cancer therapies, in lung and liver.

Session III

a. Therapeutic Inhalation Products: Issues and Strategies

b. Drug Absorption and Distribution (Session 2)

SESSION IIIa

Evaluation of Bioequivalence of Locally Acting Nasal Drug Products in the US

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Safety and effectiveness of multisource (generic) drugs is based on documentation of their bioequivalence (BE) to the corresponding reference listed drugs (RLDs). Determination of *in vivo* BE of multisource locally acting nasal drug products is complicated, in part, due to the intended delivery of drugs directly to the local site of action independent of absorption into the systemic circulation. The conventional (pharmacokinetic) methods of documentation of BE, though used for determination of equivalent systemic exposure from nasal delivery, are not sufficient to establishing equivalence in local delivery. It is because (1) drug moiety(ies) detected in the systemic circulation may have very little to no relevance to delivery of the emitted dose to the local site, and (2) measurable levels of drug in the circulation may actually appear subsequent to the onset of local effects. Consequently, documentation of equivalence in local delivery is based on clinical endpoint studies. Metering devices are principal determinants of drug delivery. Variations in the design of the components of the drug delivery systems can have a profound effect on *in vivo* performance of the drug product. Therefore the FDA recommendations for documentation of BE of nasal drug products also solicit evidence to support equivalent performance of metering devices, based on a number of *in vitro* tests that characterize the drug delivery and spray plumes. These tests are designed to determine the potency (single actuation content), priming and re-priming (where applicable), particle size and/or droplet size distributions, and plume shape (spray pattern and plume geometry). This presentation will provide a discussion on the above scientific paradigms for establishment of BE of nasal drug products.

Formulation Development of Intranasal Products: Listening to the Voice of the Customer

Amyn P. Sayani, Pharmaceutical Development Department, GlaxoSmithKline, Mississauga, ON, Canada

Nasal delivery remains an attractive route of delivery for the local and systemic delivery of drugs, as evidenced by the relatively high value of nasal products currently on the market. With an increased emphasis on delivering products that offer value to the customer, nasal delivery offers an opportunity to create products that better meet patient expectations for therapeutic efficacy, onset and duration of action. Key to successful nasal product development is acquiring a thorough understanding of nasal delivery and the various factors that affect it. This presentation will review a systematic approach to formulation development and optimization in relation to a molecule's physicochemical properties and therapeutic action. This will be linked to careful device selection and application from a portfolio of innovative devices that are currently available in the marketplace, as well as designing a device that meets the patient's needs. Novel analytical methodologies and approaches towards automation where possible will also be discussed. Parameters such as optimal spray characteristics, formulation components and drug particle characteristics, vis-à-vis current regulatory requirements, and their relation to dose deposition and efficacy will be presented. Nasal product development offers unique opportunities to link product development to therapeutic efficacy and customer compliance.

Methacholine Challenge: A Tale of Two Methods

Donald W Cockcroft, Division of Respirology, Critical Care and Sleep Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Methacholine inhalation challenges are used to measure airway hyperresponsiveness (AHR) both for clinical and for research purposes. There are many different methods and the need for adequate standardization has been recognized after the fact. The ATS published detailed methods for two of the more common procedures (AJRCCM 2000;161:309). While the tidal breathing method delivers twice as much aerosol per concentration as the five-breath dosimeter method, the two were felt to give equivalent results based on one old study, probably because of improved deposition and retention of aerosol by the dosimeter method. We compared the two methods by ATS standards in 40 subjects and demonstrated that the dosimeter method on average produced less response (higher

methacholine PC_{20}). However, this difference occurred primarily in the subjects with mild airway hyperresponsiveness (PC_{20} > 1-2 mg/mL) and several of our subjects with mild asthma had normal dosimeter methacholine challenges (Chest 2005;127:839). We further went on to show that in asthmatics with mild AHR and negative dosimeter challenges, an identical dosimeter challenge with half lung capacity inhalations demonstrated a positive result demonstrating that the deep inhalations mandated by the ATS method were actually *bronchoprotective* (JACI 2004;114:517). We further went on to demonstrate that we could also produce negative methacholine challenges in some mild AHR asthmatics by introducing five deep inhalations into the tidal breathing method (Chest 2005;128:4018). A more recent study has demonstrated that the loss of the deep inhalation bronchoprotective effect is associated not only with a greater degree of baseline AHR, but also an increase in the airway inflammation as measured by sputum eosinophils and by exhaled nitric oxide. This series of studies has also demonstrated that the two methods, despite the dose difference, give comparable results in subjects with moderate or greater AHR. The important messages are as follows:

1. The five-breath dosimeter method probably does result in improved deposition and retention of aerosol and, therefore, maximizes the dose and effect of inhaled medication.
2. When inhaling a bronchoconstrictor such as methacholine, this improved delivery is counteracted by the well-recognized bronchodilator and bronchoprotector effect of maximal lung inflation seen in normal subjects and subjects with mild asthma/mild AHR.
3. This reduces the diagnostic *sensitivity* of the dosimeter methacholine method resulting in a large number of false negative tests; this is important since it is the high sensitivity that is the hallmark of the diagnostic methacholine challenge.

Second-Entry Inhaled Corticosteroids - Inflammation is the Key

Irvin Mayers, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

There are multiple issues related to determining whether generic metered dose inhalers for the delivery of respiratory medications are in fact equivalent to a given reference product. These products act locally and therefore measuring an index of systemic absorption is not an ideal surrogate. Locally acting, inhaled corticosteroid (ICS) drugs remain unavailable worldwide, even though these drugs already exist in multiple inhaler formats in some markets. Health Canada through the Scientific Advisory Committee on Respiratory & Allergy Therapies (SAC-RAT), developed a novel approach to approval of ICS second entry products for the treatment of asthma.

Asthma is complex syndrome with differing clinical phenotypes. There is also a broad range of asthma clinical responsiveness to ICS. Some of the variability in response to medications such as corticosteroids can be described by specific genetic differences while others can be ascribed to environmental factors (e.g. cigarette smoke). Because of this variability, it is difficult to use clinical criteria to therapeutic equivalence. One feature that can define asthma is airway inflammation and this feature has been used to evaluate drug efficacy in other settings. The proposed Health Canada guidance includes:

1. Population: Subjects should have stable asthma and be steroid-naïve. They should demonstrate at least 3% eosinophils measured in their sputum. The diagnosis of asthma should be based on recognized standardized guideline recommendations.
2. Study Duration: A parallel design should be at least three-weeks in duration to allow sufficient time for a plateau to be achieved. Any other experimental design or inflammatory marker would require submitted justification.
3. Choice of Dose: A single dose consisting of the lowest dose marketed by the sponsor of the Canadian reference product should be used.
4. Clinical Efficacy Criteria: A change of at least 50% in sputum eosinophil counts between active treatment and placebo is needed.
5. Therapeutic Equivalence Criteria: To demonstrate the bioequivalence of the test (T) product compared with the reference (R) product, the 90% C.I. of the T/R ratio of mean change from baseline eosinophil count should be within 80-125% based on log transformed data or untransformed data.

SESSION IIIb

Molecular Biopharmaceutics: A New Era

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During the past fifteen years the Pharmaceutical and the Biopharmaceutical Sciences have undergone a revolution. Biopharmaceutics in particular has progressed from simple, empirical fitting of drug absorption profiles (incorrectly) based on plasma levels (1960-70's), to mechanistic biochemical-physiological models. In particular the Biopharmaceutics Classification System (BCS) and the Biopharmaceutics Drug Distribution System (BDDCS) bring molecular mechanistic insight to the Absorption process (BCS) and the Distribution, Metabolism and Elimination processed (BDDCS) i.e. all of ADME. The implications and utility of these systems is pervasive, having implications from drug discovery all the way to drug product regulation and from insilico drug design to dissolution standards for ensuring drug product efficacy. This pervasive role of biopharmaceutical science will be highlighted with aspects of insilico oral drug design on the one hand and with drug dissolution standards on the other.

Drug Targeting for Improved Distribution: Cyclosporin A

Afsaneh Lavasanifar, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Recent advancements in proteomics and genomic have resulted in tremendous growth in our knowledge on many cellular processes involved in the progression and establishment of many diseases. As a result, several new possible "molecular targets" in diseased condition have been identified and a number of new potential potent drugs that can either specifically interact or affect the expression of these "molecular targets" have been developed. However, the new target specific drugs were mostly unsuccessful in finding their way out of research labs into clinical practice due to low water solubility, poor bioavailability or short biological half life. Drug that were able to pass these obstacles, faced problem of non-selectivity for the diseased tissues and caused life threatening side effects in patients. These observations emphasize the clear need for the development of "magic shot guns" that can solubilize, protect and selectively deliver their specific drug cargo to diseased tissue destination. Our research group has reported on the development of a polymer based nano-formulation for an old, but potent and versatile drug, i.e., cyclosporine A (CyA). The polymeric nano-formulation of CyA has been able to increase the water solubilized levels of CyA up to 100 fold and change the normal biodistribution of CyA redirecting the drug from major sites of CyA toxicity, i.e., kidneys, towards blood circulation. The changes in the biodistribution of CyA by its polymeric nano-formation have resulted in reduced CyA induced nephrotoxicity in comparison to commercial formulation of this drug, i.e., Sandimmune®. Besides, the polymeric nano-formulation of CyA is found to be equally effective as Sandimmune® in overcoming P-glycoprotein mediated resistance to doxorubicin in breast tumor models *in vitro*. The results of this study points to a potential for properly designed nano-technology devices in increasing the therapeutic index of many existing and emerging drugs through modifications in the biological disposition and tissue selective delivery of the encapsulated drug.

Acrylate Nanogels and Their Microcapsular Devices for Peroral Peptide Delivery

Hideki Ichikawa, Faculty of Pharmaceutical Sciences & Cooperative Research Center of Life Sciences, Kobe Gakuin University, Kobe, Japan

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of biologically active peptides for therapeutic use. In general these peptides have relatively short half-lives, requiring appropriate controlled delivery systems for better therapy. Controlled release of peptides is, however, not as easy as that of conventional drugs because their large molecular size is much more dramatic in hindering the diffusion and release from polymeric devices. Poor chemical stability of the peptides is another issue in the manufacturing of delivery devices. From this perspective, we have been developing several types of particulate devices fabricated by acrylate-

based nanogels or their assemblies into microcapsular membranes for controlled peptide-drug delivery. These include thermo- and pH-responsive core-shell nanogel particles (CSNPs) for oral peptide delivery and delayed-release microcapsules (DRMCs) for colon-specific peptide delivery. The CSNPs composed of thermo-sensitive poly(*N*-isopropyl acrylamide) core and poly(methacrylic acid-*g*-ethylene glycol) shell layer could be prepared by the photo-initiated dispersion polymerization. This nanogel device is designed so as to demonstrate specific functions such as peptide loading at a peptide-friendly condition, i.e., neutral pH and low temperature in aqueous media, protection of incorporated peptides from harsh environment in the stomach, muco-adhesion, inhibition of proteolytic enzymes and cellular tight junction opening effect for enhancing peptide transport through the epithelial cell monolayers in the small intestine. The CSNPs possessed peptide-loading capacity of around 20% in maximum, an ability to release peptide drugs and to adhere to mucin layer in a pH-dependent manner, and the peptide-transport enhancing effect against the Caco-2 cell monolayers. Meanwhile, the DRMCs containing insulin were prepared by an air-suspension coating process using a newly synthesized nanogel particle of poly(ethyl acrylate-methyl methacrylate-2-hydroxyehtyl methacrylate). The microencapsulated insulin was pH-independently released in a delayed manner. Such a nature led to the significantly reduced glucose level followed by the peroral administration of the insulin-containing DRMCs to beagle dogs. In this presentation, therapeutic strategies and basic performances including swelling behaviours, peptide-loading capacity, controlled-release functions for several types of peptide drugs, in vitro cellular interaction, and in vivo absorption studies of peptide drugs in these nano- and micro-particulate devices will be discussed in detail.

Hyperlipidemia and Drug Distribution

Dion Brocks, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

In pharmacokinetic theory, it is typically thought that only the unbound fraction of drug in plasma is capable of leaving the blood compartment and being taken up by the cells comprising the tissues. For this reason the best concentration vs. effect relationships for drugs are typically ascribed not to total drug concentrations, but rather to the unbound drug concentrations. A potential complication is thrust upon this theory when one is dealing with a drug that possesses physiochemical qualities that promote its association with lipoproteins. Unlike other plasma drug binding proteins such as albumin and α_1-acid glycoprotein, lipoproteins possess a complex structure containing polar and apolar lipids, and specialized proteins called apoproteins. Each of the various classes of lipoproteins, classified by their biological origin, fate, composition and density, are capable of differential association of certain drugs within plasma. The apoproteins content is important in mediating some enzyme activities and lipid transport activities, and from the perspective of drug disposition, important for recognition of lipoprotein receptor mediated uptake of lipoprotein remnant particles by tissues. This provides for a possible lipoprotein-bound directed, or targeted, tissue uptake of drug. In the presence of a hyperlipidemic state, this might potentially result in an increase in drug uptake into certain tissues rich in lipoprotein receptors, despite the overall decrease in unbound fraction that is imparted on the drug in plasma. To date this aspect of drug disposition, which has the potential to modify therapeutic effectiveness, has received little attention. In this presentation existing data regarding drug disposition and effect in hyperlipidemia will be presented. In addition, recent data in a hyperlipidemic rat model generated from our laboratory, utilizing the model drugs cyclosporine A, amiodarone and halofantrine will be discussed. The data collected thus far indicates that indeed, increased lipoprotein binding in hyperlipidemia may influence drug disposition in a manner unexpected based solely on the change in unbound fraction in plasma.

Session IV

a. Personalized Therapeutic Strategies in the Treatment of Cancer: From Discovery to Implementation

b. Drug Absorption and Distribution (Session 3)

SESSION IVa

Cell-Based Assays for Targeted Discovery of Anticancer Agents

Michel Roberge, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada.

The taxane and vinca alkaloid families of microtubule-targeting agents (MTAs) are clinically important anticancer drugs. They block cell cycle progression at mitosis by activating the spindle assembly checkpoint. These drugs have dose-limiting toxicities, notably neutropenia and neurotoxicity, that restrict their clinical utility because they also target spindle microtubules of normal dividing cells such as bone marrow cells and microtubules of non-dividing cells such as neurons. Furthermore, many cancers are inherently resistant to these drugs or become so during treatment. To overcome these difficulties, we established a cell-based screen for chemicals that block cells at mitosis, screened crude extracts from natural sources, and identified a number of novel compounds, including some that target proteins other than microtubules or that are active against drug-resistant tumour cells. The mechanism responsible for arrest at mitosis after treatment with MTAs has been well studied but the molecular pathways through which tumour cells die after mitotic arrest are not well defined. MTA-treated cells can remain blocked at mitosis for prolonged periods of time but they eventually escape arrest and enter interphase without having completed nuclear or cell division – a process termed mitotic slippage. It has been hypothesized that mitotic slippage is an important determinant of cell death after exposure to MTAs. To address this hypothesis, we have developed an assay for mitotic slippage and used it to screen compound collections for chemicals that stimulate mitotic slippage. We will describe active chemicals identified to date, the mechanisms whereby they force cells to escape mitotic arrest and the consequences for cell cycle progression, ploidy, survival and proliferation. Overall, these studies are generating a "tool kit" of chemicals that arrest cells at mitosis in novel ways or that manipulate mitotic slippage. These studies will lead to a better understanding of how to use mitotic arrest to clinical advantage. They may reveal new targets for mitotic arrest and strategies to enhance the activity of antimitotic drugs. They may also reveal lead structures for the development of new therapeutic agents.

Novel Therapeutic Strategies: Optimizing Combination Therapy with Anti-Neoplastic Agents

Marcel Bally, BC Cancer Research Centre, Vancouver, BC, Canada

In Canada, there will be an estimated 166,400 new cases of cancer in 2008. During this year it is also estimated that 73,800 deaths from cancer will occur. The five year survival rates for patients with pancreatic, lung and liver cancer remain very poor and those indications where treatments are resulting in significant increases in life expectancy patients often develop recurrent disease that is very aggressive and resistant to available therapies. Effective treatment strategies are desperately needed for those cancer patients that have relapsed following treatment of the primary disease or for those patients that have an aggressive treatment unresponsive disease at first diagnosis. A common goal of many investigators is to identify and characterize specific molecular changes in cancer and to improve care by targeting these changes. If one believes that molecularly heterogeneous cancers are amenable to classification based on shared alterations of pathways that modulate growth, survival, movement or differentiation, then it will be possible to classify targets for more effective and less toxic therapies. If this paradigm is true then there is an urgent need to develop pharmaceuticals that will simultaneously impact multiple therapeutic targets. Many investigators are using cell based assays to measure drug interactions that combine to produce significant therapeutic effects and to identify drugs that act synergistically when used in combination. However, very few investigators are trying to establish strategies for the development of drug combinations or drug combination products that will capture the therapeutic benefits of these synergistic combinations and have a reasonable potential for being developed for human clinical trials over the next five years. In this presentation, strategies to identify drug combinations and drug combination products that are pharmaceutically viable will be discussed.

Impact of Inflammation on Drug Disposition in Cancer: Repression of Hepatic CYP3A and Drug Transporter Genes by Tumour-derived Cytokines

Graham Robertson[1], Marina Kacevska[1,2], Rohini Sharma[1,2], Chris Liddle[2], Stephen Clarke[1], Cancer Pharmacology Unit, ANZAC Research Institute, Concord Hospital, Sydney, NSW, Australia.

A major challenge in pharmacogenomics is the narrow therapeutic index of anti-cancer treatments, as toxicity due to variable drug clearance is a common cause of treatment failure. CYP3A4 is the major pathway of human drug metabolism, responsible for clearance of over half of all anti-cancer agents. While many studies have sought to establish links between CYP3A4 polymorphisms and altered pharmacokinetics of anti-cancer agents, genetic differences do not appear to account for variable CYP3A-mediated drug metabolism in cancer. Recently, we observed that reduced CYP3A4 activity in cancer patients was correlated to the degree of systemic inflammation, resulting in enhanced toxicity of anti-cancer drugs. To investigate hepatic expression of the human *CYP3A4* gene in the presence of cancer, we used a transgenic mouse model of the *CYP3A4* regulatory region attached to *lacZ.* The EHS sarcoma was injected into the hind limb of transgenic *CYP3A4/lacZ* mice. Markers of inflammation included cytokine levels and hepatic expression of Serum Amyloid protein P (SAP), the major mouse acute phase protein. Assessment of CYP3A function by the midazolam sleep test showed that midazolam-induced anaesthesia was increased in tumour-bearing mice. Hepatic CYP3A4 and mouse Cyp3a11 expression was decreased in tumour-bearing animals (2), while SAP expression was increased 8 fold. The mRNA expression levels of other CYPs and drug transporters including Mrp2, Mrp3, Oatp2, Oatpc, MDR2 and Bcrp was also down-regulated in the liver. Protein profiling by advanced MS-based techniques show that multiple CYPs, phase II enzyme and drug transporters are reduced. We have also observed reduced CYP3A and drug transporters associated with inflammation in mice with breast, melanoma and colon explant tumours showing that repression of drug clearance pathways is a feature of diverse cancers. Evidence for the involvement of IL-6 included raised serum concentrations, increased phospho-STAT3 protein, activated MAP kinases and SOCS3 mRNA levels in the liver. Blocking IL-6 action with a specific antibody partially restored Cyp3a11 levels. CONCLUSIONS This study has shown for the first time that cancer-associated inflammation transcriptionally represses drug clearance pathways. The transgenic mouse model of human CYP3A4 regulation, coupled with explant tumour models, permits pre-clinical testing of anti-inflammatory interventions aimed at making cancer treatment safer and more effective. Reduced levels of hepatic drug transporters, as opposed to enhanced expression within cancer cells, has significant implications for the management of multi-drug resistance in tumours. Consideration of the impact of tumour-derived cytokines on the pharmacokinetics of anti-cancer drugs may make a significant contribution to individualised treatment and better outcomes for cancer patients.

Novel Drug Delivery Strategies: Localized and Sustained Intraperitoneal Delivery of Taxanes for the Treatment of Ovarian Cancer

Christine Allen, Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada

Ovarian cancer is the 5th leading cause of cancer death in women. Often referred to as the silent killer, more than 75% of patients are diagnosed at advanced stages with peritoneal metastases. Standard therapy for advanced ovarian cancer consists of aggressive cytoreductive surgery followed by intermittent, intravenous (IV) cycles of a taxane combined with a platinum compound. Treatment with these agents is accompanied by cumulative and/or irreversible toxicities which are mostly attributed to systemic drug exposure. While these tumors are initially responsive to chemotherapy most patients relapse eventually with drug resistant disease resulting in 5 year survival rates of only 15-35% with mean survival times of only 2-4 years. Therefore, at present the prognosis for women with ovarian cancer is poor and new approaches for treatment must be developed. Various delivery strategies to improve tumor responsiveness to chemotherapy have been proposed. One approach is intraperitoneal (IP) delivery which exposes tumors and peritoneal metastases to high local concentrations of chemotherapy while reducing systemic exposure. Recently, the National Cancer Institute issued a recommendation that IP chemotherapy should be included in treatment regimens for advanced ovarian cancer. Our group (Allen and Piquette-Miller Laboratories) has designed an implantable film from chitosan and specific phospholipids for IP delivery of paclitaxel. The composition of the film was optimized in order to achieve the desired physico-chemical, mechanical and drug release properties. The mechanism for the release of paclitaxel from this system includes swelling followed by

degradation and then macromolecular relaxation as the dominant processes. The chitosan-lipid film was found to be biocompatible both *in vitro* in SKOV-3 cells and *in vivo* in CD-1 mice. Importantly, fibrous encapsulation of the implant was not detected in mice up to 4 weeks after surgical implantation in the peritoneal cavity. As well, the chitosan-lipid implant system was found to increase the maximum tolerated dose of paclitaxel and enhance anti-tumor efficacy in a human tumor xenograft model of ovarian cancer, in comparison to a commercial paclitaxel formulation (Taxol®, Bristol-Myers Squibb). Most recently, sustained treatment with this formulation was shown to significantly reduce tumor repopulation, in comparison to treatment with bolus, intermittent administration of paclitaxel. Overall, this biodegradable, biocompatible system that provides sustained and controlled release of paclitaxel may be of clinical significance for the treatment of advanced ovarian cancer.

SESSION IVb

Advances in Respiratory Drug Delivery

Warren Finlay, Department of Mechanical Engineering, University of Alberta, Edmonton, AB, Canada

Delivery of drugs to the respiratory tract as inhaled aerosols is common in the treatment of pulmonary disease, and has recently received renewed interest as a means of systemic delivery. However, several challenging aspects of aerosol delivery remain unsolved, and advances in these areas will be discussed as follows. Conventional antibiotic treatments are frequently delivered as inhaled aerosols to treat respiratory infections, particularly in cystic fibrosis patients. However, microbial resistance is a recurring theme during extended treatment of such patients. As an alternative therapy, bacteriophages have proven successful in this regard but have never been examined in a modern aerosol therapy context. Recent work with nebulized bacteriophages specific to Burkholder cepacia complex suggests this is a promising avenue for inclusion in the arsenal against resistant respiratory infections. The use of microparticles containing drug-loaded nanoparticles has shown promise as a means of enhancing drug efficacy, but dispersion of agglomerated nanoparticles upon delivery as dry powder aerosols with this approach is not certain. For this reason, inhalable nanoparticles with an active release mechanism have been developed. In vitro cell culture tests show that doxorubicin bound to such nanoparticles is more effective against lung cancer cells than free doxorubicin, and in vivo studies demonstrate that the survival of mice can be improved if doxorubicin loaded nanoparticles are administered as powders via the pulmonary route of administration. Localized physical targeting of delivery with inhaled aerosols is not possible with conventional delivery technologies. However, recent *in vitro* work has achieved localized targeting of inhaled aerosols by using smart high aspect ratio particles (SHARPs) that respond to remotely applied magnetic fields. Alignment of drug-loaded SHARPs with local magnetic field lines results in enhanced drug delivery in the region of applied magnetic field. Such an approach may be useful in enhancing drug delivery to localized sites in the lung, for example in the treatment of lung cancer. The use of magnetic resonance imaging (MRI) to quantitatively assay the localized deposition of such particles by taking advantage of the effect of their magnetic susceptibility as a contrast agent shows exciting promise as a way to assess the success of such targeting.

Pulmonary Drug Delivery System: Inhalable Nanocomposite Particles for the Treatment of Lung Cancer

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The nanocomposite particles having an aerodynamic diameter of 2.5 □m composed of sugar and anti cancer drug-loaded PLGA nanoparticles (5 %) can reach deep in the lungs, and they are decomposed into anti cancer drug-loaded PLGA nanoparticles in the alveoli. Sugar was used as a binder of PLGA nanoparticles to be nanocomposite particles and is soluble in alveolar lining fluid. The primary nanoparticles with the diameter of 200 nm containing anti cancer drug were prepared by using a probe sonicator. And then they were spray dried with the carrier material, trehalose. The nanocomposite particles were immediately decomposed into nanoparticles when added to distilled water. Almost 60 % of anti cancer drug was released from the nanocomposite particles with constant ratio during 15 days in PBS solution at 37°C. The anti cancer activity was evaluated using the lung cancer cell line A549. The

value of ED_{50} 3 days after the addition of the drugs was about 30 nM for free anti cancer drug and 10 nM for PLGA nanocomposite particles loaded with 5 % anti cancer drug. Much higher concentration of anti cancer drug was observed in the lungs than that in blood, when the nanocomposite particles were pulmonary administered.

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The Biopharmaceutics Drug Classification System and Quality by Design

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

The introduction of the Biopharmaceutics Drug Classification System (BCS) in 1995 by Amidon et al. changed the way we look at orally administered pharmaceuticals today. The BCS is a scientific classification for orally administered molecules to characterize their potential biopharmaceutical performance. The basic BCS parameters and their application in formulation development will be reviewed. There were several suggestions how to improve the BCS in the past decade; the suggested improvements will be explained. The BCS classification was adopted by the FDA and other regulatory agencies world wide. The BCS is used by the FDA to develop a regulatory frame work to allow *in vitro* test rather than clinical studies to demonstrate bioequivalence between different products and/or formulation changes. Regulatory and scientific criteria for biowaivers will be discussed. Quality by Design (QbD) is a systematic approach in product development that begins with predefined objectives and process understanding. Sound science and quality risk management are the center piece of QbD. The principles of the BCS can be considered as the foundation of QbD. Possible future regulatory frame works for biowaivers based either on the BCS, in vitro in vivo correlations or QbD will be shown.

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 a new guidance is being developed by Health Canada. It will combine bioequivalence guidances Part A and Part B as well as Report C recommendations from the Expert Advisory Committee. Major areas for consultation include: How to deal with outliers?, Do we need to continue requesting potency corrected analysis?, When should food studies be requested?, and Do we need steady state studies for modified release products? This talk will outline the proposed changes to Health Canada's approach to bioequivalence determination.

Speaker Biographies

Christine Allen
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 40 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 for Pharmaceutical Sciences/GlaxoSmithKline Early Career Award. She is the host and Chair of the 6th International Nanomedicine and Drug Delivery Symposium to be held in Toronto from October 18 – 19, 2008.

Gordon L. Amidon
College of Pharmacy, University of Michigan, Ann Arbor, Michigan 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. He 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 270 published papers and 300 abstracts, 17 US patents, contributed chapters to over 30 books and monographs and is co-editor of five books. Dr. 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. Dr. Amidon is the editor of the American Chemical Society Journal, *Molecular Pharmaceutics*. He is the director of the Center for Molecular Drug Targeting at the College of Pharmacy, The University of Michigan.

Marcel B. Bally
Department of Advanced Therapeutics (BC Cancer Agency); Department of Pathology and Laboratory Medicine, Faculty of Medicine (UBC); Faculty of Pharmaceutical Sciences (UBC) and, Division of Drug Evaluation-Pharmacology (Centre for Drug Research and Development), Vancouver, BC

Dr. Bally focuses 100% of his time on the development and characterization of novel lipid-based nanopharmaceuticals (LNs) for use in the treatment of cancer. The diversity of his funded projects and collaborations reflect opportunities to purse an assortment of lipid-based drug carrier technologies as well as explore a number of therapeutic strategies involving the use of established approved anti-cancer drugs, novel molecularly targeted drugs (e.g. small molecules, antisense oligonucleotides, siRNA), gene therapy and immunotherapy. Dr. Bally is the Co-leader (along with Dr. Karen Gelmon) of the Department of Advanced Therapeutics, a translational research department within the B C Cancer Agency. The Department of Advanced

Therapeutics operates the Investigational Drug Program (IDP), a business unit that supports a number of academic and industrial collaborations, serves as a GLP and GMP manufacturing and analytical lab for early stage products destined for use in GLP toxicology studies and early phase clinical trials, hopefully to be conducted at the BC Cancer Agency. Dr. Bally's efforts have led to a generation of novel drug carrier technologies with the specific goal of improving the therapeutic activity of drugs useful in the treatment of cancer. The research has resulted, in part, in 5-spin-off companies (Lipex Biomembranes, Canadian Liposome Co., Northern Lipids Inc., Inex Pharmaceuticals, and Celator Technologies). This work also has resulted in over 150 peer reviewed manuscripts and 40 patents/patent applications and one approved anticancer drug.

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 and Metabolism. 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.

Dion Brocks
Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Dion Brocks is an Associate Professor and Assistant Dean of undergraduate studies in the Faculty of Pharmacy and Pharmaceutical Sciences at the University of Alberta. Prior to commencing his academic career, Dr. Brocks worked as in hospital and community pharmacies in Alberta. After receipt of his M.Pharm. degree in 1986 under the supervision of Dr. Y.K. Tam, he served as Clinical Coordinator at the Rockyview Hospital Pharmacy department in Calgary. He received his doctoral degree in Pharmaceutical Sciences under the supervision of Dr. F. Jamali, specializing in Pharmacokinetics, from the University of Alberta in 1993. He was employed by the Department of Drug Metabolism and Pharmacokinetics at SmithKline Beecham Pharmaceutical from 1993 to late 1995, after which he took a position as Assistant Professor at the University of Saskatchewan. In 1998, he joined the faculty at the Western University of Health Sciences in Pomona CA (suburban Los Angeles) as Associate Professor of Pharmaceutical Sciences. He taught pharmacokinetics and pursued his research interests there involving stereoselective pharmacokinetics of halofantrine until January 2002, when he returned to his alma mater as Associate Professor of Pharmaceutical Sciences. Dr. Brocks has published over 66 peer-reviewed manuscripts related to pharmacokinetics, and has authored numerous published abstracts and some book chapters. He is a member of the editorial board of the Journal of Clinical Pharmacology and Biopharmaceutics and Drug Disposition, and is Managing Editor of the Journal of Pharmacy & Pharmaceutical Sciences. His current research interest is directed towards understanding the influence of lipids and hyperlipidemia on the kinetics and dynamics of lipoprotein-bound drugs (funded by CIHR), and the relationship between CYP1A1 and drug-induced pulmonary toxicity.

Ajai K. Chaudhary
Drug Disposition, Cancer and Endocrine, Lilly Research Laboratory, Indianapolis, IN, USA

Dr. Ajai Chaudhary is currently a head in Department of Drug Disposition at Eli Lilly and Company. His group supports the drug disposition activities in areas of oncology and endocrine during drug discovery, development and registration. His responsibilities include allocating and prioritizing resources, consulting on scientific and business issues, budgeting and approval of expenses, communicating and implementing corporate priorities and policies, implementing departmental or divisional strategy, and streamlining business practices. He is also responsible for Drug Disposition's strategy for external networks within Asia. Ajai received his bachelor's, Master's and Ph.D. degrees in Pharmaceutical Sciences from Panjab University, Chandigarh, India. Prior to joining Lilly in 1998, Ajai had worked in various research positions at University of Saskatchewan, Indiana University School of Medicine, Vanderbilt University, and at Taylor Technologies, Inc. Ajai was also the Associate Director of Mass Spectrometry Core Facilities at Vanderbilt University. He has also held adjunct faculty positions at Vanderbilt University and University of Pennsylvania. Ajai has over 45 publications in the scientific literature and has made more than 100 presentations at various international forums.

Diana Shu-Lian Chow
College of Pharmacy, University of Houston, Houston, TX, USA

Dr. Chow (B.S. in Pharmacy, National Taiwan University; M.S. in Natural Product Chemistry, Ohio State University; Ph.D. in Pharmaceutics, University of British Columbia, Vancouver, Canada) is Associate Professor of Pharmaceutics and Director of Institute for Drug Education and Research in College of Pharmacy, University of Houston, and Adjunct Associate Professor of Immunology in M.D. Anderson Cancer Center, University of Texas Her research endeavour has been focused on the development of innovative drug delivery systems for anti-cancer and neuroprotective agents, using liposomal, co-solvency, nanoemulsion, nanosuspension, microsphere and transdermal approaches. Dr. Chow has trained 17 Ph.D. and 5 M.S. graduates and 6 postdoctoral associates, all well placed in academia, the pharmaceutical industry, and FDA. Her research achievements include numerous publications, patents, and the development of a commercial product, Busulfex® for preparative regimen of bone marrow and stem cell transplantations. The product significantly improves the grafting success, minimizing hepatic toxicity from 20 to 3% and reducing fatal rate from 40% in 3 months to 6% in 1 year.Dr. Chow received American Association for Cancer Research (AACR-MSI) Faculty Scholar Awards in 2005 and 2006. Dr. Chow was invited plenary speakers in national and international conferences, including the 2000 Conference of the New York Academy of Sciences on Camptothecins. She has been a grant reviewer for FDA, NIDA and NIAID/NIH since 1997, and currently serves on Editorial Board of the Journal of Drug Development and Industrial Pharmacy (DDIP) and as a reviewer for most major pharmaceutical journals, and a consultant to hospitals in the Texas Medical Center and to pharmaceutical companies nationwide.

Donald W. Cockcroft
Division of Respiratory Medicine, Department of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Dr. Cockcroft graduated with a BSc in Chemistry from the University of British Columbia in 1967, and an MD from the same university in 1970. Specialty training included two years of General Internal Medicine and one year of Allergy and Clinical Immunology at the Royal Victoria Hospital (McGill University) in Montreal, one year of Clinical Respiratory Medicine at St. Paul's Hospital (UBC) in Vancouver, and two years of research experience in Chest & Allergy (asthma) at St. Joseph's Hospital (McMaster University) in Hamilton, Ontario under the direction of Dr FE Hargreave. Specialty (fellowship) in General Internal Medicine was obtained from the Royal College of Physicians and Surgeons of Canada in 1975. He has been on faculty in Respiratory Medicine at the University of Saskatchewan since 1977. Dr. Cockcroft is a Fellow of the American Academy of Allergy, Asthma and Clinical Immunology, a Fellow of the American College of Chest Physicians and a member of the Canadian Society of Clinical Investigation, Canadian Thoracic Society, Canadian Society of Allergy and Clinical Immunology, the American Thoracic Society, the Canadian Medical Association, the Saskatchewan Medical Association, the Saskatchewan College of Physicians and Surgeons, the Canadian College of Physicians and Surgeons, the American College of Allergy, Asthma and Immunology, European Respiratory Society, and is past-President of the Canadian Thoracic Society (1998-1999). Dr. Cockcroft has presented approximately 230 invited lectures at

international, national, and regional meetings. He has co-authored more than 193 research papers, as well as in excess of 93 review articles and/or book chapters. His current interests include asthma and research into both allergic and nonallergic airway responsiveness. He also grows prize-winning lilies.

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 140 research papers including over 30 on the principles and 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.

Warren Finlay
Department of Mechanical Engineering, University of Alberta, Edmonton, AB, Canada

Dr. Finlay is the founding director of the Aerosol Research Laboratory of Alberta (ARLA). He obtained M. Sc. (1984) and B. Sc. (1983) degrees in Electrical Engineering (Engineering Physics) from the University of Alberta and a Ph.D. in Mechanical Engineering from Stanford University (1987). He joined the University of Alberta as an Assistant Professor in 1987, and was promoted to the rank of Associate Professor in 1990 and full Professor in 1993. He has published over 95 highly respected journal articles on pharmaceutical aerosols, as well as more traditional engineering fields, and has 16 patents and patent applications in more than 20 countries. He is a reviewer for more than 25 archival journals, as well as being an editor and editorial advisory board member for several journals. He has taught widely at the University of Alberta at both the undergraduate and graduate levels in Engineering, Pharmacy and Medicine. He is the author of a book entitled "The Mechanics of Inhaled Pharmaceuticals Aerosols: An Introduction", published by Academic Press, and is the recipient of various academic awards for outstanding achievement, including the Alberta Summit Research Excellence Award, a Killam Annual Professorship, the Young Investigators Award from the International Society for Aerosols in Medicine, a McCalla Research Professorship and the Birks Gold Medal in Engineering, among others. He leads an outstanding team of students engaged in aerosol research at the University of Alberta and works extensively with companies around the world on drug delivery to the lung.

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

Dr. Gallicano is Director, Biopharmaceutics, 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 67 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 on pharmacokinetic and pharmacostatistical aspects of drug interactions, as well as chaired or co-chaired international meetings on these topics.

Fabio Garofolo
Bioanalytical Services, Algorithme Pharma Inc., Laval (Montreal), QC, Canada

Dr. Garofolo has been working in the CRO & pharma bioanalytical and LC-MS analytical fields for more than 19 years (1989-2008). At present, he is leading, as Vice-President, the Bioanalytical Services at Algorithme Pharma Inc., a world leader CRO offering a complete range of clinical development and bioanalytical services to the pharmaceutical industry. Dr. Garofolo has always been heavily involved and committed to working as a volunteer for pharmaceutical non-profit organizations with the mission to promote the interactions among industrial, academic and regulatory bodies to provide education and forums for discussion in the pharmaceutical practices. Currently, he is the regulatory advisor, official instructor, and honorary member of Calibration and Validation Group (CVG). He is also a Steering Committee member of AAPS CRO Focus Group and a reviewer of abstracts for Annual AAPS Meeting. Capitalizing on an extensive occupational background within his specialization field, Dr. Garofolo has been teaching courses, and chairing a variety of events in the pharmaceutical field since 1995. Indeed, Dr. Garofolo is the founder and chair of the Canadian LC-MS Group. The most well-known scientific meetings of this group are the Annual Symposia, which reached more than 400 attendees during its 6th edition in 2007. Dr. Garofolo was also the organizer of the Canadian Workshop on Recent Issues in GLP Bioanalysis on May 2007 and on April 2008 in Montreal (a 1-day full immersion workshop for CROs and pharmaceutical companies involved in providing bioanalytical data for bioavailability, bioequivalence, pharmacokinetic, and comparability studies). He is also working in proposing an AAPS workshop on generic, biotech and big Pharma outsourcing strategies. **Career Steps:** Dr. Garofolo is presently the Vice-President of Bioanalytical Services at Algorithme Pharma Inc. From 2003 to 2005, he was the Bioanalysis & Pharmacokinetic Head at Vicuron Pharmaceuticals Inc. From 2000 to 2003, he was the Technical Manager at Lilly & Co. From 1998 to 2000, he was the Laboratory Director at Biovail CRO. From 1994-1998, he was the Laboratory Director for R&D Dept. at IAF. **Accomplishments at a glance:** Dr. Garofolo has 69 publications & presentations in international conferences. He has developed 142 innovative GLP bioanalytical and analytical methods. He designed and invented 3 innovative bioanalytical approaches. He is the author and instructor of 37 courses. He is the recipient of the following awards: Lilly & Co Achievement Award (2001); Lilly & Co Global Award (2002); Lilly & Co Emmerson Award (2003).
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David J. Hauss
Development Metabolism and Pharmacokinetics at Bristol-Myers Squibb Company, Princeton, NJ, USA

Dr. Hauss is a Principal Scientist in Development Metabolism and Pharmacokinetics at Bristol-Myers Squibb Company. He serves on multiple development teams where he is responsible for the conduct and interpretation of non-clinical pharmacokinetic and toxicokinetic studies in support of drug and formulation development. Dr. Hauss holds a Bachelor's degree in Pharmacy and a Doctoral degree in Pharmaceutics, both from the Philadelphia College of Pharmacy & Science (University of Sciences in Philadelphia). Dr. Hauss has 19 years of experience in the pharmaceutical industry in the areas of drug delivery, pharmaceutical preformulation, Phase I clinical dosage form development, and non-clinical and clinical pharmacokinetics of small molecule and protein pharmaceuticals. Dr. Hauss has published original research and presented internationally in the areas of drug delivery, drug disposition, analysis of drugs in biological fluids, surgical technique, and physical pharmacy. He edited a book entitled, 'Oral Lipid-based Formulations: Enhancing the Bioavailability of Poorly Water-Soluble Drugs', which was published in 2007 in the Informa Healthcare (formerly Marcel Dekker) Drugs and the Pharmaceutical Sciences series. He serves as a reviewer for several refereed scientific journals, including *Journal of Pharmaceutical Sciences* and *Pharmaceutical Research* and maintains academic ties with The Danish University of Pharmaceutical Sciences (Denmark) and Monash University (Australia) where he has served as either a graduate thesis reviewer or invited lecturer. Dr. Hauss is an active member of the American Association of Pharmaceutical Scientists, where he founded focus groups on Lipid-Based Drug Delivery Systems and on Data Mining. From 1994 until 2004, he was a member of the PERI teaching faculty, with whom he taught a bi-annual course in Basic Pharmacodynamics.

Hideki Ichikawa
Faculty of Pharmaceutical Sciences and Cooperative Research Center of Life Sciences, Kobe Gakuin University, Kobe, Japan

Dr. Ichikawa is an associate professor at the Faculty of Pharmaceutical Sciences, Kobe Gakuin University. He obtained his BS (pharmacy), MS (pharmaceutics) and Ph.D. (pharmaceutics) degrees from Kobe Gakuin University in 1988, 1990 and 1994, respectively. After the working experience in a pharmaceutical company in 1990-1992, he joined the faculty at Kobe Gakuin University in 1992. He also worked with Prof. Nicholas A. Peppas at the University of Texas at Austin as a visiting scientist for his sabbatical base in 1998-1999. His research interests include multi-functional nanogel-based systems and their application to oral peptide delivery and atom-delivery for neutron-capture therapy of cancer; fine particle coating technologies using an air suspension spray-coating process; a dry-particle coating process based on a twin-screwed kneader; and material engineering of microcapsular controlled-release systems for colon-specific peptide delivery, thermo-sensitive drug delivery, cancer chemo-embolization therapy and fast-dissolving micro-systems for poorly water-soluble drugs. He is the author of 55 refereed articles, 11 review articles and 17 book chapters. He has also 9 JP patents and some of them are being applied to the manufacturing processes of pharmaceutical preparations. Currently he is a principal investigator of 20 research projects funded by government, pharmaceutical industries, academia, and clinical fields. Due to his leading contributions to pharmaceutical science and particle technology, he has been recognized by several awards, i.e., the 9th Distinguished Paper Award from the Academy of Pharmaceutical Science and Technology, Japan for his article published in *J. Drug Del. Sci. Tech.*, the 18th Jyotaki Award from the Society of Powder Technology, Japan, and the Hortatory Research Award from Hosokawa Powder Technology Foundations. Dr. Ichikawa has served as the editorial boards of several journals such as Current Drug Delivery and Advanced Powder Technology, and a member of the organizing committee of many international and domestic congresses. He teaches pharmaceutics, physical pharmacy, physical chemistry and medical bionanotechnology.

Fakhreddin Jamali
Faculty of Pharmacy and Pharmaceutical Sciences, 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 Pharmaceutical. Sciences, 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 of anti-rheumatic, analgesic and cardiovascular drugs, and toxicology of nonsteroidal anti-inflammatory drugs. He has published over 190 refereed articles and 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 been appointed is a Fellow of American Assoc. Pharm. Sci. He has received the Killam Professorship, McKeen Cattel Memorial Award of the American College of Clin. Pharmacol., the McCalla Professorship of the University of Alberta, the McNeil Award of Association of Canadian Faculties of Pharmacy, 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.cspsCanada). Has served as associate editor or editorial board of several journals. He teaches pharmacokinetics and is involved in pharmacy curriculum development.

Jim Kapron
Thermo Fisher Scientific, Ottawa, ON, Canada

Dr. Kapron (Ph.D., University of Alberta, Edmonton, Canada; B.Sc. McMaster University, Hamilton, Canada) began his career at Advion BioSciences contributing to the bioanalysis and new technologies teams. He worked to develop FAIMS, first at Ionalytics and then at Thermo Fisher Scientific, where he has been actively engaged in the marketing department ever since. He volunteers at Health Canada on the Enhanced Review Capacity Initiative

(ERCI-IACE). When he is not paddling his prospector canoe, he may be found applying novel technologies to solve challenging bioanalytical problems.

Deanna L. Kroetz
Department of Biopharmaceutical Sciences, School of Pharmacy, University of California San Francisco, San Francisco, CA, USA

Deanna Kroetz received her B.S. degree in Pharmacy from Ohio State University and her Ph.D. in Pharmaceutics from the University of Washington. Dr. Kroetz was a PRAT Fellow in the Laboratory of Molecular Carcinogenesis at the National Cancer Institute under the mentorship of Dr. Frank Gonzalez before joining the faculty at the University of California San Francisco. Her research interests are broadly in the area of drug metabolism, drug transport and pharmacogenetics. She is the Co-PI of the Pharmacogenetics of Membrane Transporters project funded by NIH as part of the Pharmacogenetics Research Network (PGRN). Current efforts in pharmacogenetics are focused on the functional effects of polymorphisms in the ABC transporters, and association of drug transporter and metabolizing enzyme polymorphisms with drug-induced toxicity. She is leading a national effort in the PGRN to establish drug toxicity surveillance mechanisms to identify cases of adverse drug events for the study of their genetic determinants. In collaboration with David Bangsberg, she is examining genetic differences in response to antiretroviral therapy in Uganda and San Francisco. Dr. Kroetz is also a member of the Pharmacology and Experimental Therapeutics committee of the Cancer and Leukemia Group B (CALGB) clinical trials network and serves as the principal investigator for several pharmacogenetics correlative studies on CALGB Phase III breast cancer studies and a retrospective pharmacogenetic analysis in AML. Dr. Kroetz received the AAPS New Investigator Award in Pharmacokinetics, Pharmacodynamics and Drug Metabolism, the Josephine Failer Award from the Ohio State University Alumni Association and the Leon Goldberg Young Investigator Award from the American Society for Clinical Pharmacology and Therapeutics. Dr. Kroetz currently serves on the editorial board of *Clinical Pharmacology and Therapeutics* and is on the Board of Directors of the American Society for Clinical Pharmacology and Therapeutics. She is actively involved in the graduate training of PhD students. Formal teaching responsibilities include pharmacokinetics to professional pharmacy students and drug metabolism and transport to graduate students in the Pharmaceutical Sciences and Pharmacogenomics graduate program.

Glen S. Kwon
School of Pharmacy, University of Wisconsin, Madison, WI, USA

Dr. Glen S. Kwon is a Professor in the School of Pharmacy at the University of Wisconsin. He received his B.S. in Chemistry in 1986 and Ph.D. in Pharmaceutics in 1991 from the University of Utah. He was a Japan Society Promotion for Science Postdoctoral Fellow at the International Center for Biomaterials Science in Tokyo, Japan from 1991 to 1993. He was an Assistant Professor in the Faculty of Pharmacy and Pharmaceutical Sciences at the University of Alberta from 1993 to 1997. He received the Jorge Heller Journal of Controlled Release/Controlled Release Society Outstanding Paper Award in 1994, National Institutes of Health FIRST Research Award in 1998, and American Association of Colleges of Pharmacy Faculty New Investigator Award in 1998. He was a Japan Society for Promotion of Science Fellow at the Institute of Advanced Biomedical Engineering and Science at Tokyo Women's Medical University in 2002. He received the Controlled Release Society Young Investigator Research Achievement Award in 2003. He is an Adjunct Professor in the Faculty of Pharmacy and Pharmaceutical Sciences at the University of Alberta. He is an Associate Editor for *J. of Pharm. & Pharmaceu. Sci.* and serves on several journal editorial boards. His research program focuses on polymers and colloids for drug, peptide/protein and non-viral gene delivery.

Afsaneh Lavasanifar
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Dr Afsaneh Lavasanifar began her academic career in the Faculty of Pharmacy and Pharmaceutical Sciences in November 2001. Her research is focused on the design and development of polymer based delivery systems that can increase solubility, modify the pharmacokinetic pattern, reduce toxicity and increase the efficacy of different therapeutic agents. The ongoing research projects in her laboratory include development of polymeric nano-carriers as systemic and regional delivery systems for cancer chemo/immunotherapy and development of stimulus responsive

nano-gels for skin regeneration and treatment of hypertrophic scarring and fibrosis. She has an active teaching program in both undergraduate and graduate levels in the area of pharmaceutics and drug delivery. Afsaneh has completed her PhD in Pharmaceutical Sciences in the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta. She also holds a Pharm. D. degree from Faculty of Pharmacy, Tehran University of Medical Sciences and has been a lecturer in Pharmaceutics in the Faculty of Pharmacy, Dr Beheshti university of Medical Sciences, Tehran, Iran before embarking graduate studies in Alberta. She has received several awards for her research contributions and teaching performance. She has been the recipient of the 2007GlaxoSmithKline/CSPS Early Career Award in Montreal meeting. Afsaneh has 36 peer reviewed published/in press manuscripts in highly ranked journals in pharmaceutical sciences, 2 book chapters, several abstracts and numerous conference presentations. She is also the main editor of a book on "Polymeric Micelles and Related Nano-Delivery Systems" currently under preparation. Afsaneh is the co-inventor in one issued patent and the main inventor in 3 other patent applications.

Lawrence J. Lesko
Office of Clinical Pharmacology, Center for Drug Evaluation and Research, FDA, Silver Spring, Maryland

Dr. Lesko has been the Director of the Office of Clinical Pharmacology since it was formed in 1995. The office has grown from 40 to over 135 reviewers and staff over a 12 year period. It is primarily responsible for early phase studies in drug development that evaluate optimal dosing, dosing adjustments in special populations (e.g., patient with renal impairment), drug interactions, pharmacogenetics and bioavailability and bioequivalence. The Office has two specialized teams: the PGx team and a pharmacometrics team. The latter conducts research on drug-disease models and is responsible for modeling of exposure-response data and simulation of clinical trials. Dr. Lesko has published over 150 peer-reviewed papers in clinical pharmacology and has presented at many national and international meetings. He is board certified in clinical pharmacology by the American Board of Clinical Pharmacology. He is a member of the Editorial Board of Clinical Pharmacology and Therapeutics and the Journal of Clinical Pharmacology. He is Past President of the American College of Clinical Pharmacology (2004-2006) and continues as a Fellow of the College. Dr. Lesko is also an Adjunct Professor at the Schools of Pharmacy at the Universities of Florida, North Carolina and Southern California respectively. He is a Divemaster certified by the Professional Association of Diving Instructors and his hobbies include scuba diving, underwater photography and motorcycle riding on his Harley Ultra Classic Electra Glide.

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

Dr. Löbenberg holds a B.Sc. in pharmacy from the Johannes Gutenberg-University in Mainz, Germany. He received his Ph.D. in pharmaceutics from the Johann Wolfgang Goethe-University in Frankfurt in 1996. He worked as post doctoral fellowship on the fundamentals of the Biopharmaceutical Drug Classification System (BSC) with Dr. Dressman and Dr. Amidon. He joined the University of Alberta in 2000. His research interests are in formulation design and dissolution testing to predict the oral performance of dosage forms. In recent years, he has consulted different pharmaceutical and nutraceutical companies in their product development and helped them to set product specifications. The application of nanotechnology for drug delivery is another major research interest. Here Dr. Löbenberg investigates the pulmonary delivery of drug loaded nanoparticles to treat diseased like lung cancer. He is the representative of the Association of Faculties of Pharmacy in Canada to the USP Convention; he is member of the USP Dietary Supplement Expert Committee for Performance Testing and member of the USP Membership Committee.

Lorelei Lutter
Life Sciences Division, CANTEST Ltd., Burnaby, BC

Ms. Lorelei Lutter is currently the Senior Director of Business Development of the Life Sciences Division of CANTEST, which specializes in pharmaceutical analysis and bioanalysis for the pharmaceutical and biotech industry. She has been with CANTEST since 2004. Ms. Lutter has over 15 years experience in the pharmaceutical and CRO industry, in both large and small companies. In her current role, Ms. Lutter is responsible for managing the commercial functions of business development for the pharmaceutical related testing business of CANTEST. Previously, Ms. Lutter was Director of Sales and Marketing at Biovail Contract Research, and Manager of Business

Development at Pharma Medica Research Inc. Prior to Pharma Medica, Ms. Lutter held scientific and managerial positions at Genpharm Pharmaceuticals and Biovail Contract Research. Ms. Lutter earned her MBA from the Schulich School of Business at York University, and a Bachelor of Science (Honours) degree in Human Biology and Nutritional Sciences the University of Toronto. She is an active member of the American Association of Pharmaceutical Scientists (AAPS). She currently serves as Secretary for the Canadian Society for Pharmaceutical Sciences (CSPS).

Kimiko Makino
Faculty of Pharmaceutical Sciences, Tokyo University of Science, Japan

Dr. Kimiko Makino is a Professor in the Faculty of Pharmaceutical Sciences at Tokyo University of Science. She received her B.S. in Pharmaceutics in 1979 and Ph.D. in Pharmaceutics in 1987 from Tokyo University of Science. She was a Postdoctoral Fellow at the University of Utah, USA, under the guidance of Prof. S. W. Kim from 1988 to 1989. She was an Assistant Professor from 1981 to 1995, Lecturer from 1995 to 1997, Associate Professor from 1997 to 2002, and is a Professor since 2002 in the Faculty of Pharmaceutical Sciences, Tokyo University of Science. Also, since 2004 she is a Professor in Center for Drug Delivery Research, Tokyo University of Science. She serves on the editorial boards of Colloids and Surfaces B: Biointerfaces. Her research program focuses on polymers and colloids for drug delivery.

Gordon McKay
College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada

Dr. Gordon McKay received his BSc and PhD degrees in biochemistry from the University of Saskatchewan. After a brief postdoctoral training period in pharmaceutical science he was appointed as a research associate and adjunct professor of pharmacy in the College of Pharmacy at the University of Saskatchewan and a principal investigator in the Drug Metabolism, Drug Disposition Research Group headed by Dr. Kamal K. Midha at this same institution. The research group received the first program grant awarded by the Medical Research Council to a College of Pharmacy and the first ever awarded to the University of Saskatchewan. The focus of this research was "Investigations towards the more efficacious use of antipsychotic drugs." This research was renewed for a total of 11 years after which the group began to focus on collaborative research with the pharmaceutical industry and has continued in this regard for a total of 28 years. Dr. McKay was awarded fellowship in the American Association of Pharmaceutical Sciences in 1994 and was one of the founding members of the Canadian Society of Pharmaceutical Sciences on whose executive he is now currently president. He is a scientific organizer for numerous scientific meetings including the Bioanaltyical Validation meetings, the Tandem Mass Spectrometry Workshops held annually for the last 20 years and BioInternational. He has served on the editorial board for J.Pharm.Sci. and has been a member of the Pharmaceutical Sciences review committee for MRC and has served on numerous University Boards and Committees. Dr. McKay has published more than 165 original scientific publications and authored more than 200 scientific presentations. Currently he is the Associate Dean of Research and Graduate Affairs in the College of Pharmacy and Nutrition. He serves as Co-chair of the University of Saskatchewan Biomedical Research Ethics Board and is a member of the Academic Health Sciences Biomedical Research Working Group. Dr. McKay is also an active consultant and chief executive officer of Pharmalytics Inc., a for profit corporate research laboratory located in the research park at the University of Saskatchewan, engaged in conducting contract research for the pharmaceutical industry throughout the world.

Irvin Mayers
Pulmonary Medicine, University of Alberta, Edmonton, AB, Canada

Dr. Mayers (MD, FRCPC) is a Professor of Medicine and Divisional Director, Pulmonary Medicine, at the University of Alberta. He completed his medical training at the University of Manitoba and completed his Pulmonary clinical and research training at University of Manitoba and University of Chicago. He moved to the University of Alberta (1994) and has been divisional director of Pulmonary Medicine (2001-current). Dr. Mayers' clinical interests include asthma, COPD and sleep medicine. He is past president of the Canadian Thoracic Society (CTS) and the current Chair of the CTS Research Committee. He has also acted as a clinical content expert for health technology assessment and Common Drug Reviews undertaken by Canadian Agencies for Drugs and

Technologies in Health (CADTH). Dr. Mayers is the Co-Director of the Alberta Strategy to Help Manage Asthma (ASTHMA project), a provincial consortium evaluating the care gaps in asthma or COPD management and then developing programs to close the care gaps. He is chair of the Health Canada Scientific Advisory Committee on Respiratory & Allergy Therapies that has recently developed drug guidances for introduction of second entry inhaled corticosteroids.

Sylvia Ng
Department of Advanced Therapeutics, BC Cancer Agency, and University of British Columia, Vancouver, BC

Dr. Sylvia Ng is a Senior Scientist in the Department of Advanced Therapeutics at the British Columbia Cancer Agency and an Assistant Professor in the Faculty of Pharmaceutical Sciences at the University of British Columbia. She obtained her BSc (1996) and MSc (1998) in Pharmacology & Therapeutics from the University of British Columbia, and completed her PhD (2002) in Medical Biophysics at the University of Toronto/Ontario Cancer Institute. Subsequently, Dr. Ng received her postdoctoral training at the National Cancer Institute, National Institutes of Health (Bethesda, MD), and at Sunnybrook & Women's College Health Sciences Centre in Toronto. Her current research focuses on: a) studying the molecular interactions between pancreatic cancer cells and cancer-associated fibroblasts and how such interactions may contribute to chemotherapy resistance in pancreatic cancer; and b) understanding the tumour microenvironmental changes induced by antiangiogenesis-based therapies at the whole tumour level and how such changes may be exploited for pancreatic cancer therapy. The overall goals of her research are to identify novel therapeutic targets and to develop more effective treatment strategies using new drugs or combinations of existing drugs for pancreatic cancer. Dr. Ng is also the leader of the British Columbia Pancreatic Cancer Research Net (www.bcpcrnet.ca), which is a network of researchers and clinicians from the British Columbia Cancer Agency, Vancouver General Hospital, and the University of British Columbia working together as a unit with the aim to improve the prognosis of pancreatic cancer patients.

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. With this work has come a strong interest in method validation from the statistics point of view. Eric obtained a BSc. in Genetics and Statistics from the University of Guelph and a MSc. in Biostatistics also from Guelph. Currently he is acting manager of the Office of Science, 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.

Tetsuya Ozeki
School of Pharmacy, Tokyo University of Pharmacy and Life Sciences (TUPLS), Tokyo, Japan

Dr. Ozeki (M.Sc and Ph.D., pharmaceutics and drug delivery/pharmaceutical technology, Tokyo University of Pharmacy and Life Sciences (TUPLS), Tokyo, Japan) is an associate professor at the School of Pharmacy, TUPLS. He joined the school of pharmacy at TUPLS in 1995 as a research associate and became an assistant professor in 1999. He worked with Dr. Valentino J. Stella in the University of Kansas as a visiting scientist from 2003 to 2004. He became an associate professor at TUPLS in 2006. His research interests are: 1. One-step preparation of nanoparticles-containing microspheres using a special spray nozzle, which includes the preparation of polymeric nano-carrier, the improvement of absorption of drugs, and the preparation of nanoparticles for inhalation therapy of tuberculosis; 2. Brain tumor therapy by sustained release microspheres in thermoreversible hydrogel using anti-cancer drugs and fusion peptide of tumor suppressor peptide-cell penetration peptide; 3. *In silico* analysis of the complexes between drugs and next-generation cyclodextrin derivatives. He has published 40 articles, has been an invited speaker at 8 international and 10 domestic conferences. He has received the Nakako Yoshida Memorial Award-Research Award, the Academy of Pharmaceutical Science and Technology, Japan (APSTJ) Global Education Seminar Presentation Award and the Grant of the APSTJ Asahi Kasei Encouragement Award of Pharmaceutics. He is a member of the Global Education Seminar Committee, the Future Vision Committee, and PR

Committee of APSTJ. He is a member of editorial board of Journal of Japan Society of Pharmaceutical Machinery and Engineering. He teaches pharmaceutical technology, applied pharmaceutics and practical training of pharmaceutics.

Xuejun Peng
Pharmaceutical Operations Division, CANTEST Ltd., Burnaby, BC

Dr. Xuejun Peng is currently the Laboratory Manager of Bioanalytical R&D Services in the Pharmaceutical Operations division at CANTEST Ltd., which specializes in GMP pharmaceutical analysis and GLP bioanalysis to support pharmaceutical and biotech companies with their drug discovery and drug development programs. He has been with CANTEST since 2006 and has taken the responsibility of Study Director/Principal Investigator for LC-MS/MS method development, validation and sample analysis for GLP studies. Previous to CANTEST, Dr. Peng had scientist research positions at QLT/University of British Columbia, MethylGene and Absorption Systems. Dr. Peng received his Bachelor (1986), Master (1989) and Ph.D. (1992) in Analytical Chemistry from Wuhan University, China, and had postdoctoral training at Xiamen University and University of British Columbia. He spent more than 15 years on ADME/DMPK related bioassays, working with pharmaceutical companies and contract research organizations. He is an active member of the American Association of Pharmaceutical Scientists (AAPS) and American Society for Mass Spectrometry (ASMS). He is the author and co-author of more than 50 publications, in journals, patents and conference presentations.

Micheline Piquette-Miller
Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada

Micheline Piquette-Miller, [PhD, University of Alberta, 1994] is a Professor at the University of Toronto, Faculty of Pharmacy. Her research specializes in the area of drug transport and molecular pharmacokinetics. Based on this research, Dr. Piquette-Miller has been the recipient of numerous national and international research awards including the prestigious *Leon I Goldberg Young Investigator Award, Rx &D Health Research Foundation Research Career Award,* the *Pfiasky Young Investigator Award,* the *University of Alberta Horizon Award* and the *Toronto Institute of Pharmaceutical Technology's Award of Research Excellence*. She is currently president of the *Canadian Society of Clinical Pharmacology*, has served on the Executive Boards for CSPS and the American Society of Clinical Pharmacology and Therapeutics (*ASCPT)*.

K. Wayne Riggs
Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada

Dr. Riggs (BSc Pharmacy, MSc pharmacokinetics, PhD pharmacokinetics, University of British Columbia, Vancouver, BC, Canada) is a professor in the Faculty of Pharmaceutical Sciences, University of British Columbia. He joined the faculty in 1989 and has served as chair of the Division of Pharmaceutics and Biopharmaceutics (1998-2000) and interim associate dean of graduate studies (2003-2004). His research interests include the detailed examination of the pharmacokinetics of placental drug transfer in both model systems and human subjects. Another major area of interest involves the investigation of the effects of single nucleotide polymorphisms in reductase genes on the efficacy/toxicity of various drugs used in the treatment of breast cancer and leukemia as well as on endogenous hormones in prostate cancer. He has published 55 peer reviewed articles, been an invited speaker at several conferences and has trained 1 MSc and 9 PhD students. Dr. Riggs has been a major contributor to curriculum development. He is a member of many graduate advisory, faculty and university committees. He has received a number of teaching awards (UBC Killam Teaching Prize, Bristol-Myers Squibb Teaching Award). He has served as a consultant and/or a member of the board of directors for a number of pharmaceutical/biotechnology companies. He is a current member of the CIHR Pharmaceutical Sciences grants review panel and the Health Canada TPP Expert Advisory Committee on Bioavailability and Bioequivalence. He also serves as a reviewer for several scientific journals.

Michel Roberge
Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Dr. Roberge (Ph.D. University of Heidelberg) is a Professor at the Faculty of Medicine, University of British Columbia. He joined the Faculty at the University of British Columbia in 1991 after postdoctoral work in the laboratories of Dr. Morton Bradbury at the University of California Davis and Dr. Susan Gasser at the Swiss Institute for Experimental Cancer Research. His research expertise is in the development of cell-based screening assays and the discovery of small molecules that target cellular processes involved in cancer, including the cell cycle and invasion. He is co-discoverer of HTI-286, an anti-tubulin agent in Phase II clinical trials. He is a co-founder of the Canadian Chemical Biology Network and of the Centre for Drug Research and Development where he is Division Head, Drug Screening. He is currently Chair of the NCIC "Experimental therapeutics, medicinal chemistry and drug development" grant review panel. He teaches biochemistry to undergraduate and graduate students.

Graham Robertson
University of Sydney and ANZAC Research Institute, Concord RG Hospital, Sydney, NSW, Australia

A/Prof Robertson is a research scientist within the University of Sydney and a Senior Research Fellow of the ANZAC Research Institute at Concord RG Hospital, NSW Australia. He is the senior scientist and group leader responsible for establishing the Cancer Pharmacology Unit and coordinating the pre-clinical research program as well as a Cancer Institute NSW translational program grant to use advanced proteomic techniques to discover protein biomarkers for colorectal cancer. Graham's research interests are: mechanisms of hepatic gene regulation; impact of tumour inflammation on drug disposition, especially human CYP3A4; role of tumour-derived cytokines in the development of cancer cachexia; regulation of metabolic pathways by nuclear receptors; development of transgenic mouse models for liver studies; pathophysiology of liver injury; discovery of biomarkers for cancer.

Naír Rodríguez-Hornedo
Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, MI, USA

Naír Rodríguez-Hornedo is Associate Professor of Pharmaceutical Sciences at the University of Michigan and was co-founder of the Pharmaceutical Engineering Program. Dr. Rodríguez has developed a research program based on a molecular-mechanistic approach, founded on the premise that the concepts of supramolecular chemistry and crystal engineering can be applied to: (i) design novel pharmaceutical materials with desirable composition, structure and properties, and (ii) to understand crystallization pathways and solid phase transformations that are important in controlling pharmaceutical processes and outcomes. In 2005 Dr. Rodríguez was awarded the Ebert Prize for the best article published in the Journal of Pharmaceutical Sciences. Dr. Rodríguez has served on the FDA Advisory Committee for Pharmaceutical Sciences. She serves on the editorial boards of the Journal of Pharmaceutical Sciences and the Encyclopedia of Pharmaceutical Technology.

Allen D. Roses
Drug Discovery Institute, Duke University Medical Center and Fuqua School of Business, and Cabernet Pharmaceuticals, Inc., Durham, NC, USA

Dr. Roses was one of the first clinical neurologists to apply molecular genetic strategies to neurological diseases. His laboratory at Duke reported the chromosomal location for more than 15 diseases, including several muscular dystrophies and Lou Gehrig's disease**.** He led the team that identified apolipoprotein E4 [APOE4] as the major susceptibility gene for common late-onset Alzheimer's disease in 1992. In 1997, when Dr. Roses left Duke University Medical Center, he was the Jefferson Pilot Professor of Neurobiology and Neurology, Founding Director of the Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Chief of the Division of Neurology, and Director of the Center for Human Genetics. Dr. Roses became Senior VP for Genetic Research at GlaxoSmithKline where he supervised the translation of the APOE4 association, finding new metabolic pathways for Alzheimer's disease, as well as new lead molecules for drug development. His laboratory teams then completed the first efficacy pharmacogenetic clinical trial -identifying responsive and non-responsive patients in a large Phase IIB clinical trial. This work in drug discovery and development led to three ongoing Phase III trials of rosiglitazone

for the treatment of AD, the first of which completes in 2009. Dr. Roses' GSK teams also performed the proof of principle experiments for identifying genetic causes of serious adverse effects from drugs. This work culminated in finding the first highly accurate predictive test for drug allergy. A pioneer in the application of whole genome analyses for several common diseases, Dr. Roses returned to Duke to initiate the Deane Drug Discovery Institute to translate exploratory research into molecules suitable for translational medicine.

Amyn P. Sayani
Pharmaceutical Development Department, GlaxoSmithKline, Mississauga, ON, Canada

Dr. Sayani joined GlaxoSmithKline Canada in 1999, and currently serves as a team leader in the Pharmaceutical Development Department, based in Mississauga, Ontario. In this role, Amyn leads a multifunctional team of analytical and pharmaceutical scientists in developing semisolid and oral dosage forms, and has also led the formulation of intranasal products. The team is involved in the development of new chemicals entities, from preclinical stages to launch. Amyn's current research activities include the development of novel topical and oral formulation approaches to enhance delivery, and also the use of acoustic signals for process end-point detection. Dr. Sayani received his Ph.D. from Rutgers University, and his background includes developing implantable and intranasal delivery systems for therapeutic peptides and proteins. Dr. Sayani has numerous publications in journals and conference proceedings, and 10 patents published. Amyn has served as a member of the CSPS Executive and serves on a graduate advisory committee at McMaster University.

Gur Jai Pal Singh
Watson Laboratories, Corona, CA, USA

Dr. Singh is a Director of Biopharmaceutics at Watson Laboratories in Corona, California, where he oversees clinical evaluation of a variety of drug products. Before joining Watson in 2006, he worked for 15 years in the Division of Bioequivalence at the US FDA. He joined the Agency as a reviewer and subsequently became a Team Leader. In addition, he was the designated Division Expert for the locally acting drug products including inhalation aerosols, metered nasal sprays and dermatologic products containing corticosteroids. His interests also included application of pharmacokinetic and pharmacodynamic (PD) modeling and computer simulations for resolution of complex bioequivalence (BE) issues. At the FDA, in addition to evaluation of BE studies on a variety of drug products, Dr. Singh specialized in the development of methods and standards for establishment of comparative in vivo and in vitro performance of locally acting drug products. He played key roles in the development of BE methodologies for albuterol metered dose inhalers, nasal sprays and dermatologic corticosteroids. He actively participated in the Agency efforts to develop methods for evaluation of comparative in vitro performance of devices used in drug products intended for oral inhalation and nasal drug delivery. He chaired the CDER PD/BE Working Group and the Dry Powder Inhaler Working Group, and was an active member of several committees which focused on resolution of complex issues and prepared the Agency guidances for documentation of in vivo BE and equivalent in vitro performance of locally acting drug products. He also participated in the Agency-sponsored clinical studies designed to gain insights for the development of novel BE methodologies. During his tenure with FDA Dr. Singh also enthusiastically participated in teaching the CDER Staff College's courses on Advanced Pharmacokinetics, Modeling and Regulatory Science. In addition, he gave a series of lectures to train the review staff for evaluation of complex BE studies on locally acting drug products, and received numerous awards for his contributions. He has given invited lectures at a number of national/international conferences, made key presentations at several Advisory Committee meetings, and participated in expert panel discussions.

John M. Seubert
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

Dr. Seubert (BSc, MSc, Simon Fraser University, Burnaby, BC; PhD (Pharmacology & Toxicology), University of Western Ontario, London, ON); Post-Doctoral Fellowship (Cardiovascular Pharmacology), National Institutes of Environmental Health Sciences (NIEHS/NIH), Research Triangle Park, NC, USA) joined the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, as an assistant professor in July 2005. He is currently a Heart and Stroke New Investigator and AHFMR health scholar, as well as the recipient of the McDonald Scholarship from Heart and Stroke Foundation of Canada. The central focus of his research is to investigate the cellular and

physiological effects of fatty acid metabolites produced by cytochrome P450 enzymes (CYP) in the cardiovascular system. In particular, the protective role of those generated from arachidonic acid, called epoxyeicosatrienoic acids (EETs). EETs are important components of many intracellular signaling pathways in both cardiac and extracardiac tissues. The research utilizes a multilevel approach to address the molecular, cellular and physiological importance of CYP enzymes and fatty acids in the heart. Presently, there are 9 members in Dr. Seubert's research group ranging from summer students to postdoctoral fellows. Academically, Dr. Seubert teaches cardiology and graduate research. Dr. Seubert serves as a reviewer for numerous scientific journals and the Heart and Stroke Foundation of Canada. He serves on the Human Research Ethics Board at the University of Alberta and scientific advisory to Health Canada. He is a member of the Cardiovascular Research Group at the University of Alberta.

Elizabeth B. Vadas
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Elizabeth B. Vadas has over 26 years of drug development expertise in the area of oral, aerosol and ophthalmic formulations of synthetic small molecules. She obtained her Ph.D. in Physical Chemistry from McGill University in Montreal and her undergraduate degree in colloid and surface chemistry in Budapest, Hungary. She spent 22 years at Merck Frosst; the Canadian subsidiary of Merck & Co. Prior to taking early retirement in 2002, Dr. Vadas was Executive Director of Pharmaceutical Research and Development at Merck Frosst. The department, under her leadership, was responsible from early compound characterization to formulation and process development of new chemical entities from phase I through phase III, including the supply of clinical trial materials and also for technical transfer from research to manufacturing. In mid-2002 Dr. Vadas decided to take early retirement to establish her own consulting company, InSciTech Inc. Currently she works with several drug discovery companies in the US, Canada and in Europe providing development expertise and support. Dr. Vadas is a Fellow of AAPS, Past President of the Canadian Society for Pharmaceutical Sciences and is an adjunct professor of pharmaceutics at the University of Montreal. She has lectured and published widely and is the recipient of a number of scientific and management awards including the CSPS Leadership Award.

Robert Young
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Robert Young earned a B.Sc. (1967) from the University of Victoria, a Ph.D. (1971) from the University of British Columbia, and continued postdoctoral studies at the Imperial College of Science and Technology, the University of Adelaide and the University of British Columbia between 1971 and 1976. From 1976 to 1977, he was a Research Associate at the Institut de Chimie des Substances Naturelles in Gif-sur-Yvette, France, and from 1977 until 2006 he worked in various capacities with Merck Frosst Canada & Co. He was Vice-President and Head of Medicinal Chemistry at the Merck Frosst Centre for Therapeutic Research until taking early retirement in 2006. He is now Merck Frosst-B.C. Discovery Chair in Pharmaceutical Genomics, Bioinformatics and Drug Discovery in the Chemistry Department at Simon Fraser University his current research is focused on the design and use of novel pharmacological probes and proof of concept molecules for the discovery and validation of new drug targets. He is also co-director of the Division of Design and Synthesis of the Center for Drug Research and Development in British Columbia and consults for a number of Pharmaceutical companies through his own company, Promorpheus Consultants Inc. Dr. Young's career has focused on the design and synthesis of novel drugs for asthma, inflammation, osteoporosis and related diseases and he is most noted for his part in the discovery of the asthma drug, Singulair™. Dr. Young's He is the author of more than 160 publications, review articles and patents. Dr. Young is a member of the Order of Canada and a Fellow of the Royal Society of Canada, and his academic and professional honours and affiliations are numerous. He was awarded a National Merit Award from the Ottawa Life Sciences Council and a Heroes of Chemistry Award from the American Chemical Society. He is a member of the Natural Sciences and Engineering Research Council of Canada and was recently recipient of the first Genome BC Leadership Award.

George Zografi
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Dr. Zografi (B.S. Pharmacy, Columbia University; M.S. and Ph.D. Pharmaceutics, University of Michigan) is the Edward Kremers Professor Emeritus of Pharmaceutical Sciences at the School of Pharmacy, University of Wisconsin-Madison. He served on the faculties of the Columbia University, College of Pharmacy (1960-1964) and the University of Michigan, College of Pharmacy (1964-1972) before joining the faculty of the University of Wisconsin-Madison in 1972. From 1975-1980 he also served as Dean of the School of Pharmacy. Dr. Zografi's research interests have been focused in two scientific areas related to drug product development: the physical chemical properties of non-crystalline solids and the surface chemistry of lipids, polymers and proteins in monolayer and bilayer systems. He has has published close to 200 scientific and educational publications, been an invited speaker at many national and international symposia and seminars, served as a consultant for many pharmaceutical companies, and has trained over 50 Ph.D. students and postdoctoral research associates. He has served on the editorial boards of: the Journal of Colloid and Interface Science; Journal of Pharmaceutical Sciences; International Journal of Pharmaceutics and Pharmaceutical Research, and on the Committee of Revision of the U.S.P (1985-1995). For his academic achievements and research, he has received a number of awards, including: the APhA Research Achievement Award for the Stimulation of Research (1988); the APhA Ebert Prize (1984 and 2001); the AAPS Dale E. Wurster Award for Pharmaceutics (1990), the AAPS Distinguished Scientist Award (1995); the AACP Volwiler Research Achievement Award (1996) and the Distinguished Educator Awards of both AACP (1989) and AAPS (2002). In 1989, he was elected to the Institute of Medicine of the National Academy of Sciences, and in 2005 he was awarded a University of Wisconsin-Madison Citation of Merit. He also received an honorary Doctor of Science degree from the Columbia University, College of Pharmacy in 1976.

Poster Presentations

Friday, May 23, 2008

Friday, May 23 2008

Biomedical Sciences

1. Regulation of the membrane expression of somatostatin receptor subtypes (SSTR 1 to 5) under tamoxifen and estradiol treatment and the effect of oxidative stress on the cell-death pathway in human breast cancer cells

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Purpose: Somatostatin is an inhibitory neuropeptide that can exert antiproliferative effects on solid breast cancer tumors. These effects are mediated by five different receptor subtypes of G-protein coupled receptors, namely SSTR1 to SSTR5. All subtypes are expressed in variable levels as membrane and cytosolic proteins in both estrogen positive MCF-7 and estrogen negative MDA-MB-231 human breast cancer cells. One desirable goal might then be to upregulate the expression of SSTRs in breast tumors. As a result, further investigation on the effects of tamoxifen and estradiol on the membrane expression of all subtypes of SSTRs in both ER+ and ER- breast cancer cell lines will provide insights to improve treatment. On the other hand, reactive oxygen species play a significant role in the induction of apoptosis and has also been shown in tumor progression. However, when oxidative stress continually builds up, the nature of cell death might be mediated by necrosis as well. Better understanding of the cell death pathway of breast cancer cells may provide valuable knowledge on the role of both somatostatin and estrogen receptors on the cell physiology under oxidative stress. **Research Methods:** The total membrane protein expression of all somtatostatin receptors in MCF-7 (ER+) and MDA-MB-231 (ER-) breast cancer cells was determined using western blot analysis. Furthermore, semiquantitive analysis using immunocytochemistry was performed to compare SSTR1-5 expression in these cells under no treatment and the treatment of 1uM of estradiol and tamoxifen, alone and in combination. To determine the nature of cell death, different concentrations of hydrogen peroxide (10nM, 0.1uM, 10uM, 100uM, and 500uM) were used to treat MCF-7 and MDA-MB-231 cells in simulation of an environment with oxidative stress. The dyes bisbenzimide H fluorochrome and propidium iodide were used to determine apoptotic and necrotic cells respectively. In addition, we analyzed the translocation of PTP1 or PTP2 from cytosole to membrane as an index of SSTR mediated apoptosis. **Results and Conclusion:** SSTR4 is the most strongly expressed receptor in both MCF-7 and MDA-MB-231 cells, whereas SSTR3 and 5 are the most weakly expressed receptors in both cell lines. Estradiol has variable effects on the expression of somatostatin receptor subtypes; its action is estrogen receptor-dependent. Tamoxifen upregulates all subtypes of somatostatin receptor expression in breast cancer cells; its action is estrogen receptor-independent. These data suggest that the role of somatostatin on tumor growth and cell proliferation, through five receptor subtypes, might depend on the presence of estrogen.

2. Pharmacokinetics study of naratriptan and effect of vagal suppression on its bioavailability in the Rat

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

Purpose: Naratriptan is a serotonin agonist that is used to treat migraine. It has been shown that migraine attacks delay the time to reach to maximum concentration (tmax), hence, likely the onset of action. It has been attributed to delayed gastric emptying due to migraine pain. The objective of this work was to study the pharmacokinetics and oral bioavailability of naratriptan in a rat model with suppressed gastrointestinal function. **Method:** Adult male Sprague Dawley rats (weight 254 ± 30g, n=3-9/group) were cannulated in the right jugular vein and allowed to recover overnight. One and two hour before naratriptan dosing, Control rats received normal saline and Treated rats received propantheline 20 mg/kg (to suppress the GI function) through intraperitoneal route. They were divided into 4 groups: Group I (Controls i.v., received i.v. bolus naratriptan in saline, n=3); Group II (Control-Powder, received naratriptan powder suspended in polyethylene glycol (P-susp.) orally, n=8); Group III (Treated-Powder, received naratriptan as P-susp. orally, n=9); Group IV (Treated-Tablet, received naratriptan as granules of commercially available formulation orally, n=3). The dose of nartriptan was 1.8 mg/kg (base equivalent). Blood samples were collected at 5, 10, 15, 30, 60, 90, 120, 180, 240, 360 and 720 min post dose. Naratriptan plasma

concentrations were measured using a LC-MS method. **Result:** Pharmacokinetic parameters were calculated and presented as below:

Parameter	unit	i.v. Control	oral		
			Control-powder	Treatment-powder	Treatment-tablet
t 1/2	hr	1.36 (0.37)	2.51 (1.07)	nd	nd
T max	hr	nd	1.91 (0.93)	4.92 (3.42) *	2.33 (3.19)
Cmax	ng/mL	nd	77.7 (34.7)	77.4 (40.7)	108.7 (6.6)
$AUC_{0-12\,h}$	ng*hr/mL	1826.9 (1019.8)	303.7 (149.9)	593.7 (363.5)	821.4 (141.05) *
$AUC_{0-\infty}$	ng*hr/mL	1839.6 (1018.3)	322.1 (156.1)	nd	nd
Vd	L/kg	2.32 (1.23)	nd	nd	nd
CL	L/hr/kg	1.16 (0.49)	nd	nd	nd
MRT	hr	0.77 (0.46)	3.72 (0.35)	6.0 (1.4) *	6.0 (0.4) *
F (partial)			0.17	0.32	0.45 *

*Significantly different form Ccontrol -Powder group. Data are presented as Mean (SD). nd; not determined

Conclusion: Naratriptan oral absorption is delayed by vegal suppression, but its partial exposure is not affected. Low to moderate total body clearance indicates that naratriptan is not highly metabolized by liver. The significantly higher bioavailability in Treatment-Tablet group and an upward trend of bioavailability in Treatment-Powder group compared with Control-Powder group can be explained by the longer residence of the drug in the gut and its availability for prolonged absorption.

3. Single Dose Pharmacokinetics and Bioavailability of Ketamine enantiomers in the Rat

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

Purpose: Ketamine is a short acting general anaethetic which is widely used through intravenous (i.v.) or intramuscular routes. The drug also has analgesic properties. The objective of this study was to assess the single dose pharmacokinetics and bioavailability of Ketamine enantiomers after iv and oral (p.o.) and i.v. administration in the rat. **Method:** Rats were catheterized at the right jugular vein for blood sample collection. Racemic ketamine was administered p.o. or i.v. at a doses of 50 and 10 mg/kg (base equivalent) (n=3-4/group), respectively. Blood sample were collected at 5, 10, 15, 30, 60, 90, 120, 180 and 240 min post dose. Ketamine enantiomers plasma concentrations were measured using an isocratic direct chiral HPLC method with a Chiralpak AD-H column, UV detection at 215 nm and propranolol as internal standard. **Result:** R and S-ketamin demonstrated retention times of 10.0 and 13.3 minutes respectively, with baseline resolutions. The sensitivity of the method was <156 ng/mL for both enantiomers. Following i.v. administration, the elimination $t_{1/2}$ was 0.69 and 0.64 h, the volume of distribution at steady state was 6.9 and 9.1 L/kg and total body clearance was 128.7 and 164.5 mL/kg/min for R and S-ketamine, respectively. The systemic clearance of the enantiomers following i.v. doses approximated the hepatic blood flow, and assuming hepatic metabolism as the main clearance route, an absolute oral bioavailability of close to zero was predicted. The observed absolute oral bioavailability values following the 50 mg/kg dose were 0.3 and 0.17 for R and S-ketamine, respectively. The time to reach maximum plasma concentration was 0.75 and 0.25 h for R and S-ketamine, respectively. **Conclusion:** Ketamine is rapidly absorbed, widely distributed and extensively cleared upon the first pass through the liver. The relatively large total body clearance compared with hepatic blood flow indicates that ketamine is highly extracted by liver. The observed bioavailability of the enantiomers following

oral doses, despite the prediction of almost zero may be attributed to saturation of the hepatic enzymes responsible for the drug metabolism after single 50 mg/kg doses.

4. Myocardial infarction Reduces response to verapamil in isoproterenol-treated rat model due to downregulation of cardiac L-type calcium channels

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Purpose: Inflammation complicates cardiovascular diseases such as hypertension, heart failure and myocardial infarction. Inflammation has been found to reduce verapamil potency in rat models and patients with rheumatoid arthritis. Similarly, we have reported that verapamil pharmacological response is diminished in post-acute myocardial infarction (post-AMI) rat model. Since myocardial damage is in part an inflammatory process, inflammation may play a role in the altered verapamil response. The purpose of this study is to investigate the mechanism of the reduced verapamil response in post-AMI rats. **Methods**: Two groups of male Sprague-Dawley rats (230-280 g) were divided into Control (n=8) and Post-AMI (n= 13). AMI was induced by injecting 2 subcutaneous daily doses of 150 mg/kg isoproterenol HCl solution in normal saline. Subcutaneous ECG leads were implanted in the animals while they were under halothane/Oxygen anesthesia. Two days following the second injection, after baseline ECG measurement, each rat was dosed with 25 mg/kg p.o. of verapamil solution and ECG measurements were taken at 0, 20, 40, 60, 80, 100, 120, 180 and 240 min post-dosing and PR-intervals were measured. Subsequently, animals were euthanized and blood samples collected for analysis of serum tumor necrosis factor α (TNF-α), interleukin 4 (IL-4), cardiac troponin I (cTnI) and nitrite (NO_2). Western blot of cardiac L-type calcium channels ($Ca_V1.2$) in rat hearts was done. **Results**: Two isoproterenol doses were capable of inducing myocardial damage in rats. This was manifested by ST-segment elevation, J-point elevation and increased serum cTnI. AMI caused a significant reduction in verapamil potency in prolonging PR-interval. The amount of the calcium channel target protein ($Ca_V1.2$) in post-AMI rats was significantly reduced as compared to control. The area under the effect curve (AUEC) of PR-interval prolongation was found to be significantly correlated to cTnI ($r=-0.6$, $p<0.05$) and the amount of calcium channel target protein ($r=0.6$, $p<0.05$). IL-4, an anti-inflammatory mediator, was also found to be significantly correlated to Emax ($r=0.8$, $p<0.05$). Serum cTnI concentration was significantly higher in rats with detectable TNF-α.

	Control	Post-AMI
AUEC (PR-interval prolongation). %*min	808±200	202±59*
Emax (Max. PR-interval prolongation) %	10.4±1.5	5.36±0.9*
Serum cTnI (ng/ml)	4.6±0.9	12.7±2.6*
Serum IL-4 (pg/ml)	19.9±9.1	9.7±4.4
Serum NO2 (μM)	28.6±8.9	61.1±32.7

*, significantly different from control ($p<0.05$)

Conclusion: Acute myocardial infarction reduces verapamil potency. This observation is parallel to that we have observed in rat models of inflammation and patients with rheumatoid arthritis. Inflammation-induced downregulation of cardiac L-type calcium channels in rat heart is a contributing factor in the reduced response of verapamil in post-AMI rat model.

5. Cloning and characterization of metabolic genes involved in the biosynthesis of L-digitoxose

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Purpose: The jadomycins are a class of antibiotics first isolated from *Streptomyces venezuelae* ISP5230 at Dalhousie University. Both jadomycin B and the jadomycin B aglycone have demonstrated cytotoxic effects, with the higher activity resulting from the glycosylated compound, jadomycin B. Through cloning of the genes and purification of the gene products involved in the biotransformation of L-digitoxose, we aim to isolate the Jad enzymes responsible for the sequential reactions in the L-digitoxose biosynthetic pathway. Isolation of these

proteins will provide a means to perform enzymatic alterations of the Jadomycin carbohydrate functionality in vitro, producing novel compounds for biological testing.

Methods:

- Specific PCR primers were designed and suspect PCR products (JadO, JadP, JadT, JadU or JadV genes), were cloned into TOPO-Blunt® vectors for sequencing.
- Verified Jad sequences were digested from the TOPO vectors and ligated into the commercial His-Tag pET-28b vector.
- The ligated products were transformed into competent *E. coli* BL21-D3 cells for protein over-expression.
- Resulting cultures were optimally grown and induced with 1M IPTG
- The resulting cell pellet was lysed by sonication
- Protein was separated through HiTrap® FPLC column binding and eluted using an imidazole buffer.
- Identification of the protein was determined through SDS-PAGE confirmation

Results:

- The DNA sequences of Jad T and Jad U were verified using the NCBI Blast® nucleotide sequence identification program.
- The Jad U protein was soluble, able to be purified and confirmed by SDS-PAGE analysis.
- The Jad T protein formed inclusion bodies upon. Inclusion body purification procedures are being examined to recover the protein in its native state.
- Verified DNA sequences for Jad O, Jad P and Jad V genes were not obtained using conventional PCR methods.
- Over-expression and isolation of products from a synthetic Jad S gene were successful.

Conclusion: Methods for purifying gene products of Jad T, O and P are currently underway. Upon isolation of these enzymes, subsequent in vitro assays can be developed to generate precursor substrates required to examine the functionality of the Jad U enzyme. Difficulties encountered with amplification of Jad O, P and V can be avoided through the use of synthetic genes. Synthetic genes may permit more efficient translation of *Streptomyces* DNA by *E.coli*, enhancing native protein folding and promoting both solubility and enzyme functionality. Enzymatic alteration of the L-digitoxose carbohydrate unit in vitro may lead to novel jadomycin antibiotics for biological testing.

6. Nonlinear stereospecific pharmacokinetics of ketoconazole in rat after oral administration of racemate

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Purpose: To study the pharmacokinetics of ketoconazole (KTZ) enantiomers in rat after oral administration of a wide dose range of KTZ racemate. **Method:** Five groups (n=4 each) of Sprague-Dawley rats were administered single oral doses of racemic KTZ over the range 10–80 mg/kg. Serial blood samples were collected over a 24 h period via surgically-placed left jugular vein cannula. Plasma was assayed for KTZ enantiomer concentrations using validated stereospecific HPLC. Enantiomeric plasma protein binding was calculated using an erythrocyte partitioning method. Stereospecific metabolism was tested by incubating the racemic drug with rat liver microsomes. **Results:** In all rats, (+)–KTZ plasma concentrations were much higher than (–)–KTZ with overall mean (+):(–) $AUC_{0-\infty}$ ratio of 2.36. Mean tmax were 1.61±0.41 h and 1.45±0.45 h for (+)– and (–)–KTZ, respectively, suggesting similar absorption rates. The mean $t_{½}$ for KTZ enantiomers showed no significant differences within doses. However, for both enantiomers with higher doses the $t_{½}$ was found to increase. The $AUC_{0-\infty}$ with escalating dose were as follows:

	Dose (mg/kg)				
$AUC_{0-\infty}$ (mg·h/L)	10	20	40	50	80
(+)–KTZ	5.59±3.63	9.70±5.06	56.8±18.4	71.8±18.7	112±37.2
(–)–KTZ	2.51±1.43	4.11±2.55	22.7±7.71	27.0±13.0	53.9±19.5
(+):(–) ratio	2.22	2.36	2.50	2.66	2.08

The mean unbound fraction of (−) enantiomer was found to be 2.9-fold higher than that of the (+) enantiomer. There was no evidence of stereospecific metabolism after microsomal incubations. **Conclusion:** In agreement with previous reports for (±)−KTZ, both KTZ enantiomers showed nonlinearity with increasing racemic doses in rat. It appears that the stereoselectivity in plasma concentrations of KTZ enantiomers can be attributed to enantiospecificity in plasma protein binding rather than metabolism in rat. Funding: CIHR MOP 67169.

7. Effects of (*E*)-2,4-diene-VPA on markers of mitochondrial function, cell death, oxidative stress, and total cellular glutathione in primary cultures of rat hepatocytes

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Purpose: Reactive metabolite formation is a potential mechanism of valproic acid (VPA)-associated hepatotoxicity. Of particular interest is (*E*)-2,4-diene-VPA, which when activated is reactive toward glutathione (GSH) and is an inducer of hepatic steatosis in the rat. The current study compared the effects of VPA, (*E*)-2,4-diene-VPA, and a 4-ene-VPA analogue (α-F-4-ene-VPA) which could not form the (*E*)-2,4-diene-VPA on markers of mitochondrial function, cell death, oxidative stress, and total cellular glutathione (GSH) in rat hepatocytes cultured in a sandwich configuration consisting of Matrigel™. **Methods:** Time course experiments with VPA treatment indicated an optimum exposure period of 24 hours for the cellular reduction of WST-1 to formazan (a marker of mitochondrial function, EC_{50} = 1.1 ± 0.4 mM) and the release of lactate dehydrogenase (LDH, a marker of necrotic cell death, EC_{50} = 13.0 ± 1.5 mM). VPA treatment for 24 hours increased the conversion of 2',7'-dichlorodihydrofluorescein diacetate to 2'7'-dichlorofluorescein (DCF, a marker of oxidative stress, EC_{50} = 12.4 ± 1.9 mM) and depleted total cellular GSH (EC_{50} = 12.9 ± 1.2 mM) in a concentration dependent manner. **Results:** Treatment of hepatocytes with an equimolar concentration (1 mM) of VPA or (*E*)-2,4-diene-VPA decreased formazan formation by 48 ± 2 % (mean ± SEM, n = 15) or 87 ± 3 % (n = 5), respectively; whereas α-F-4-ene-VPA had no effect. Treatment of hepatocytes with an equimolar concentration (12 mM) of VPA or (*E*)-2,4-diene-VPA increased LDH release by 64 ± 2 % (mean ± SEM, n = 8) or 155 ± 1 % (n = 4), respectively; increased DCF formation by 100 ± 7 % (mean ± SEM, n = 11) or 330 ± 32 % (n = 5), respectively; and decreased GSH by 53 ± 5 % (mean ± SEM, n = 9) or 94 ± 3 % (n = 4), respectively. In contrast, the effects of α-F-4-ene-VPA were equivalent to the control. Dose response experiments with (*E*)-2,4-diene-VPA treatment indicated that the EC_{50} values were approximately 0.3 mM for the WST-1, LDH, and DCF assays and 0.1 mM for the decrease of GSH. **Conclusion:** Our model of sandwich-cultured hepatocytes allows the evaluation of the activities of VPA analogues with respect to the induction of oxidative stress and cellular toxicity. We report the novel observation that (*E*)-2,4-diene-VPA is a potent and toxic metabolite in this model. This supports previous findings indicating the reactivity of (*E*)-2,4-diene-VPA. [Supported by the Canadian Institutes of Health Research-MOP-13744]

8. Live microencapsulated *Lactobacillus fermentum* 11976 can be used to augment ferulic acid in the gastrointestinal tract: Preparation and in-vitro analysis

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Purpose: The physiologic importance of ferulic acid (FA), notably its chemotherapeutic properties, depends upon its availability for absorption and subsequent interaction with target tissues. The concentration of partially released FA (from dietary sources) in the gut is too low to act as a chemopreventive agent. The present study aims to investigate the enhancement of FA, by supplemented feruloyl esterase (FAE) activity, in the small intestine by use of orally ingested microencapsulated FAE producing lactic acid bacteria in a computer controlled, simulated human gastro-intestinal (GI) model. **Methods:** Microencapsulation of *Lactobacillus fermentum* 11976 in alginate-poly-L-lysine-alginate (APA) membrane was carried out using an Inotech Encapsulator. APA microencapsulated *L. fermentum* 11976 cells were exposed to simulated gastric conditions for 1 hour with mechanical shaking. Subsequently, they were transferred to simulated intestinal fluid for 10 hours in a simulated GI model to determine

the de-esterifying activity of the encapsulated FAE producing *L. fermentum* 11976. The simulated intestinal fluid was supplemented with 1.33mM ethyl ferulate substrate. The real time FAE activity of the microencapsulated FAE producing bacteria was investigated using high-performance liquid chromatography. **Results:** *L. fermentum* 11976 were encapsulated in an alginate-poly-L-lysine-alginate APA membrane microcapsule (mean capsule diameter was 602 $\pm$ 30 μm) containing, on an average, 10^{12} cfu/mL of bacteria. *L. fermentum* 11976 FAE^+ microcapsules de-esterified ethyl ferulate ($P < 0.001$) at a significantly greater rate than sham (empty) microcapsules. The average amount of FA liberated from ethyl ferulate was 28.0 $\pm$ 0.8 micrograms FA/g microcapsule/h for *L. fermentum* 11976 FAE^+ microcapsules and 0.1 $\pm$ 0.02 micrograms FA/g microcapsule/h for empty microcapsules. Maximal activity was reached within three hours with 28.0 $\pm$ 0.8 micrograms FA released/g microcapsule/h. After the fourth hour, there was a gradual decrease in the FAE activity of the microencapsulated cells. At the tenth hour, FAE activity had somewhat stabilized at 24.2$\pm$ 0.6 micrograms FA released/g microcapsule. Viable counts of the microencapsulated *L. fermentum* 11976 FAE^+ cells showed an increase from pre-treatment values of 10.9 log cfu/ml to 12.3 log cfu/ml when exposed to simulated intestinal fluid over a 10 hour period. The APA microcapsules were found to remain intact at the end of the incubation period. **Conclusion:** This study shows that microencapsulated *L. fermentum* 11976 cells can efficiently break down a ferulic acid containing substrate, and establishes the biotechnological basis for their use in supplementing the bioavailability of dietary ferulic acid in the intestine.

9. Diltiazem and Silymarin Enhance Hepatocyte Viability

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Purpose: To investigate the hepatoprotective role of d-diltiazem, silymarin and their combination in Chang and PLC hepatocytes. Hepatoprotection was assessed by analyzing the drugs' antioxidant activity, cell viability and inhibitory potency to the pro-apoptotic protein Bax. **Methods:** Hepatocytes (Chang and PLC) were treated with d-diltiazem (2.5 or 10μM) and/or silymarin (10 or 1000 μg/L) for 24 hrs at 37°C followed by half hour incubation with H_2O_2 (400μM) to initiate oxidative stress. The dichlorofluorescein (DCF) assay was used to assess the extent of reactive oxidative species (ROS) in the liver cells. The effect of silymarin and d-diltiazem on the cellular proliferation was determined by the MTT assay. ATP changes by d-diltiazem and silymarin were measured using bioluminescent assay. Western blot and RT-PCR were used to determine Bax expression. **Results:** Compared to control, d-diltiazem and silymarin statistically attenuated DCF fluorescences and reduced Bax expression while promoted cell viability and mitochondrial ATP content. Combining diltiazem with silymarin further protected hepatocytes compared to use of individual drugs. **Conclusions:** Diltiazem and silymarin are associated with significant hepatoprotective properties. We conclude that the combination of silymarin and d-diltiazem provides further antioxidant protective effects than d-diltiazem alone in hepatocytes.

10. Modulation of cytochrome p450 (cyp) gene expression in rats during isoproterenol-induced cardiac hypertrophy

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Purpose: The aim of the present study was to investigate the effect of isoproterenol-induced cardiac hypertrophy on the expression of multiple cytochrome P450 (CYP) genes in the heart, liver, kidney, and lung of male Sprague Dawley rats. **Methods:** Cardiac hypertrophy was induced by daily intra-peritoneal injections of 5 mg/kg isoproterenol for seven days. Animals were euthanized 24 hours after the last injection. Thereafter, the heart, liver, kidney, and lung were harvested and the total tissue RNA was isolated. The expression of hypertrophic marker, atrial natriuretic peptide (ANP) and different CYP genes were determined by real time-polymerase chain reaction (RT-PCR). **Results:** Our results showed that CYP1A1, CYP1B1, CYP2B1, CYP2C11, CYP2E1, CYP2J3 and CYP4A1 genes are constitutively expressed in all tissues at different levels. Isoproterenol-induced cardiac hypertrophy significantly increased the heart to body weight ratio as well as the hypertrophic marker, ANP by 34% and 10 fold, respectively. In addition, there was a significant induction of CYP1A1 and CYP1B1 by 2 and 5 fold,

respectively in hypertrophied heart but not in other tissues. On other hand, CYP2C11 and CYP2E1 were significantly reduced in hypertrophied heart by 2 and 7 fold, respectively but not in other tissues. CYP2B1, CYP2J3, and CYP4A1 gene expression were not significantly altered in all tissues during isoproterenol-induced cardiac hypertrophy. **Conclusion:** Isoproterenol-induced cardiac hypertrophy selectively modulates the gene expression of CYP1A1, CYP1B1, CYP2C11, and CYP2E1 in the heart, suggesting their role in the development of cardiac hypertrophy. **Acknowledgements:** This work was supported by a grant from the Heart and Stroke Foundation of Alberta, NWT, and Nunavut to A.O.S.E. B.N.M.Z. is the recipient of Egyptian Government Scholarship.

11. Induction of Cytochrome P450 1a1 (Cyp1a1) by Broccoli-derived Sulforaphane

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Purpose: Sulforaphane (SFN) is considered to be the major anti-carcinogenic component in broccoli. It is believed that SFN exerts its effect through the induction of phase II detoxifying enzymes such as NAD(P)H:quinone oxidoreductase 1 (Nqo1) and GST enzymes. Nevertheless, its effect on phase I enzymes is still a matter of debate. Therefore, we examined the effect of SFN on the expression of the aryl hydrocarbon receptor (AhR)-regulated gene, cytochrome P450 1a1 (Cyp1a1), an enzyme known to play an important role in the chemical activation of xenobiotics to carcinogenic derivatives. **Methods:** Murine hepatoma Hepa 1c1c7 cells were incubated with different concentrations of SFN (1 – 10 μM). The cytotoxicity of SFN was assessed using the MTT assay. Cyp1a1 mRNA expression was measured using real-time polymerase chain reaction. Cyp1a1 protein expression and catalytic activity levels were measured using Western blot analysis, and Cyp1a1-dependent 7-ethoxyresorufin *O*-deethylase (EROD) activity, respectively. SFN effect on Cyp1a1 mRNA decay was assessed using actinomycin D-(Act-D) chase experiment. **Results:** Our results showed that SFN had no apparent cellular toxicity effects at all concentrations tested (1 – 10 μM). In addition SFN increased the Cyp1a1 mRNA expression in a time- and dose-dependent manner. The increase in Cyp1a1 mRNA occurred as early as 1h post-treatment, implying a transcriptional activation. The RNA synthesis inhibitor, Act-D (5 μg/ml), completely blocked the Cyp1a1 induction by SFN (5 μM), indicating a requirement of *de novo* RNA synthesis through transcriptional activation. Consequentially, Cyp1a1 protein expression and catalytic activity levels were significantly increased in Hepa 1c1c7 cells in response to SFN. Looking at the post-transcriptional level, SFN did not alter the Cyp1a1 mRNA stability, suggesting a lack of post-transcriptional mechanism in the induction of Cyp1a1 by SFN. **Conclusion:** This study provides the first demonstration that SFN can directly induce Cyp1a1 gene expression through a transcriptional mechanism. These results raise the possibility that SFN may activate the pro-carcinogens to carcinogenic metabolites. **Acknowledgements:** This work was supported by the Natural Sciences and Engineering Council of Canada (NSERC) Grant RGPIN 250139 to A.O.S.E.

Drug Delivery and Pharmaceutical Technology

12. Resveratrol analog trans 3,4,5,4'-tetramethoxystilbene (DMU-212) modulates multiple cellular targets in breast cancer cells

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Purpose: Resveratrol is a well-known chemopreventive and chemotherapeutic agent. Among all of the resveratrol analogs synthesized, 3,4,5,4'-tetramethoxystilbene (DMU-212) shows high activity and selectivity against various cancer cell types. The objective of this study is to investigate the anti-cancer activity of DMU-212 and its underlying mechanisms in MDA-MB-435 and MCF-7 human breast cancer cells. **Methods:** The effects of DMU-212 on cell viability, cell cycle, Stat3 activation, and microtubule dynamic were investigated using MTT assay, cell cycle analysis, Western blot, tubulin polymerization assay, respectively, in MDA-MB-435 and MCF-7 human

breast cancer cells. **Results:** DMU-212 exerted a potent growth inhibition, giving IC_{50} of 9.9 and 63.8 μM at 48 h after treatment in MDA-MB-435 and MCF-7 cell lines, respectively. Further studies demonstrated that DMU-212 acted via multiple cellular targets. DMU-212 induced predominantly G2/M arrest in both cell lines, reduced the expression of multiple anti-apoptotic proteins significantly and resulted in an appreciable apoptosis, inhibited Stat3 phosphorylation, and led to a significant increase in tubulin polymerization. The higher sensitivity to DMU-122 in MDA-MB-435 correlated with the more prominent effects seen in these parameters in this cell line, as compared to MCF7. **Conclusion:** DMU-212, the novel stilbene derivative, demonstrated potent anti-tumor effects, which are likely owing to its modulation of multiple cellular targets.

13. The self-association of amphiphilic block copolymers to polymeric micelles and vesicles

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Purpose: To investigate the self-assembly of poly(ethylene oxide)-*b*-poly(ε–caprolactone) (PEO-*b*-PCL) block copolymers of different PCL content and under different assembly conditions into polymeric micelles and/or vesicles by transmission electron microscopy (TEM) and atomic force microscopy (AFM). **Methods:** Co-solvent evaporation method was performed to prepare nanoaggregates from block copolymers consisting of PEO-*b*-PCL with an average PEO molecular weight of 5000 $gmol^{-1}$ and 5000, 13000 and 24000 $gmol^{-1}$ of PCL. Block copolymers of different molecular weights were dissolved in acetone separately and added to water at two different acetone:water ratios of 1:2 and 1:6. For TEM studies, phosphotungestic acid ($H_3PW_{12}O_{40}$, PTA, 2% (v/v)) was used as the negative staining solution to characterize the nano-aggregates at a polymer concentration of 1 mg/mL. Further characterization was performed by AFM, where one drop of properly diluted polymeric solution was deposited on a fresh mica surface (flogopite, $KMg_3AlSi_3O_{10}(OH)_2$) . *In situ* imaging of the adsorbed polymeric solution layers on the mica surface was performed with a Nanoscope AFM and cantilevers with an integral silicon tip (OMCL-AC160TS-W2). **Results:** TEM and AFM images provided a direct evidence for the formation of micellar and/or unilamellar vesicular structures within the range of 100 nm from self assembly of PEO-*b*-PCL block copolymers. Based on TEM and AFM studies, pure polymeric micellar population was only obtained for PEO-*b*-PCL having 5000 g/mol^{-1} of PCL at 1:2 acetone: water ratio. Co-exsistance of micelles and vesicles was revealed for other samples prepared using higher PCL molecular weights at either 1:2 or 1:6 acetone:water ratios. **Conclusion:** Block copolymers with higher PCL molecular weights tend to assemble to non-uniform nano-aggregate populations consisting of polymeric micelles and vesicles.

14. Multifunctional polymeric micellar drug conjugates for tumor specific and pH triggered intracellular drug delivery

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Purpose: Tumor cell and tumor angiogenic vasculature overexpress integrin αvβ3 which can be used for targeted chemotherapy. The objective of this research was to develop RGD-functionalized biodegradable polymeric micelles with conjugated anticancer drugs, which can target αvβ3 in tumor and tumor angiogenic vasculature and selectively release their conjugated drug by pH-triggered mechanism in their target site for active tumor targeting. **Methods:** Self-associating amphiphilic block copolymer-drug conjugates consisting of cRGDfK-poly(ethylene oxide)-*block*-poly(ε-caprolactone-hydrazone-doxorubicin) (cRGDfK-PEO-*b*-P(CL-Hyd-DOX) were synthesized by installing the specific integrin-targeted ligand, i.e. cRGDfK at the end of the shell-forming PEO chain and conjugating the anticancer drug, doxorubicin (DOX) to the side chain of the biodegradable core-forming poly(ε-caprolactone) segment through an acid-cleavable hydrazone bond. The resulted multifunctional micelles were evaluated for their pH-triggered drug release, cellular uptake and cytotoxicity in human breast cancer MDA-MB435/LCC6 wild type ($MDA\text{-}MB435/LCC6^{WT}$) and P-glycoprotein expressing multi-drug resistant type ($MDA\text{-}MB435/LCC6^{MDR}$) cells. **Results:** cRGDfK-PEO-*b*-P(CL-Hyd-DOX) micelles were successfully prepared. The polymeric micellar drug conjugate did not show any sign of DOX release in physiological pH of 7.4, but illustrated acidic pH triggered release of DOX at pH 5.0. Compared to acetal-PEO-*b*-P(CL-Hyd-DOX) micelles, cRGDfK decorated polymeric micellar DOX conjugates showed increased cellular uptake and higher cytotoxocity in both wild type and MDR

cells which overexpress integrin receptors αvβ3. **Conclusions:** The combination of two targeting strategies in prepared multifunctional polymeric micelles, i.e., enhanced uptake of cRGDfK polymeric micelles by metastatic tumor cells that over-express αvβ3 integrin as well as acidic pH-triggered DOX release induced by the acid sensitive hydrazone linker is a feasible approach. This strategy is shown to improve the intracellular delivery of the conjugated DOX in target tumor cells, *in vitro*. Multifunctional polymeric micellar drug conjugates prepared under this study are expected to increase the tumor specificity and targeting efficiency of DOX after systemic administration *in vivo.*

15. Polymeric nano-carriers for the solubilization and delivery of P-glycoprotein inhibitor, PSC 833 (Valspodar)

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Purpose: To investigate the potential of polymeric micelles based on poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) and poly(ethylene oxide)-*block*-poly(α-benzyl carboxylate-ε -caprolactone) (PEO-*b*-PBCL) for the solubilization and delivery of P-glycoprotein (p-gp) inhibitor, PSC 833. **Methods:** PEO-*b*-PCL and PEO-*b*-PBCL block copolymers having identical PEO molecular weights (5000 g mol^{-1}) and different molecular weights of PCL (between 5000-13000 g/mol^{-1}) or PBCL (between 8000-25000 g/mol^{-1}) were synthesized and assembled into polymeric nano-carrires using a co-solvent evaporation method. Prepared micelles were characterized for their average diameter, thermodynamic stability, core viscosity, and PSC 833 encapsulation. Morphology of self-assembled structures was investigated by both transmission electron microscopy (TEM) and atomic force microscopy (AFM). A new liquid chromatography/mass spectrometry (LC/MS) method of assay was developed and used for quantification of encapsulated PSC 833. **Results:** The developed LC/MS method was validated and exhibited excellent performance in terms of short run time of analysis (10 min/sample), simplicity of sample preparation, precision and accuracy. In rat plasma (0.1 mL), the linearity was achieved over the range of 10-5000 ng/mL (R^2>0.99) with a lower limit of quantification (LLQ) of 10 ng/mL. For the concentrations above the LLQ, the intraday and interday CV% was equal or less than 15%, and mean error% was less than 10%. Among different core-forming blocks, optimum solubilization was achieved by utilizing polymeric nano-carrier having a PCL block of 13,000 g mol^{-1} or a PBCL block of 24,000 g mol^{-1}. PSC 833, which is practically insoluble in water, reached an aqueous solubility of 2.8 and 1.7 mg/mL in the presence of PEO_{5000}-*b*-PCL_{13000} and PEO_{5000}-*b*-$PBCL_{24000}$ micelles, respectively. All the synthesized PEO-*b*-PBCL block copolymers formed micelles at low concentrations in the range of $2.3\text{-}6.2\times10^{-2}$ μM compared to critical micellar concentrations for their PEO-*b*-PCL counterparts, which were in the range of $1.7\text{-}18 \times 10^{-2}$ μM. The average diameter of PEO-*b*-PBCL nano-aggregates was in the range of 74-104 nm compared to an average diameter of 40-100 nm for PEO-*b*-PCL nanocarriers. The TEM and AFM studies revealed self-assembly of PEO-*b*-PBCL block copolymers to polymeric micelles except for PEO_{5000}-*b*-$PBCL_{15000}$ and PEO_{5000}-*b*-$PBCL_{24000,}$ which have shown a sub population consisting of polymeric vesicles. **Conclusion:** Our results point to a great potential for PEO-*b*-PBCL nano-carriers for efficient solubilization and delivery of PSC 833.

16. The Effect of Compression Forces on the Stability of Dibasic Calcium Phosphate Dihydrate Tablets in the Presence of Glutamic Acid Hydrochloride monitored by Isothermal Microcalorimetry

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Purpose: This study was designed to investigate the ability of Isothermal microcalorimetry (ITMC) to detect any incompatibility reaction between Glutamic Acid Hydrochloride (GAHCL), as an acidic model drug, and Dibasic Calcium Phosphate Dihydrate DCPD. The influence of compression forces on the solid state compatibility of DCPD in the presence of GAHCL was studied including the impact of tablet relaxation by monitoring the total heat flow over the experiment time. **Methods**: Batches *control* powder, which was GAHCL free, and test powder, contain 1% of GAHCL, were prepared. Tablets were prepared by using Increasing compression forces starting from 0.5 and 1 to 1.5. Total heat flow of each experiment was calculated and the time required to reach maximum heat

flow was determined (Tmax). Moreover, the increase of powder's content of water was also determined. **Results:** The total heat flow of test powder was small and comprised an exothermic and endothermic phases. Moreover, an increase in water content was detected in the *test* powder over control and individual powder. The exothermic phase was totally missing in the heat flow of the test powder prepared using anhydrous dibasic calcium phosphate. The heat flows of all tablets' type were huge when compared with the test powder. The total heat flow and the Tmax were correlated with the compression force applied. Furthermore, a correlation between the relaxation heat flow and the exothermic phase in the total heat flow of each tablet type, at the first six hours, was also observed. **Conclusion:** The present study identified an exothermic and endothermic solid state reaction between GAHCL and DCPD. The detected incompatibility can be linked to a neutralization reaction between the GAHCL and DCPD on the particle surface followed by the loss of crystalline water of DCPD triggered by a local increase in humidity. The study showed that the solid state reaction is influenced by compression forces. Moreover, the relaxation energy correlates with the magnitude of the solid state reaction between GAHCL and DCPD over the first 6 hours.

17. Low-Surfactant Microemulsions for Enhanced Topical Delivery of Poorly Soluble Drugs

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Purpose: To develop low-surfactant microemulsions for enhanced topical delivery of poorly soluble drugs. **Method**: Five different microemulsions were prepared using various oil/surfactant systems. Pseudo-ternary phase diagrams were constructed to obtain the optimum concentration of components, and solubility of a model drug in each formulation was investigated. Three formulations were carried forward for further investigations based on solubilities. Thickening agents were evaluated to increase the skin applicability of the microemulsions. Rheological profiles of each formulation were extensively investigated to evaluate performance criteria such as applicability, processibility, and thermal stability. Formulations were evaluated for physical stability using temperature cycling (5-40°C ramps), accelerated conditions, and centrifugation. *In vitro* release rates of each formulation were determined using Franz diffusion cells. **Results**: The selected microemulsions showed substantially higher solubilizing capacity for the model drug than conventional formulations. Generally, Xanthan gum and Carbopol formulations exhibited excellent and fair rheological properties, respectively. Colloidal silica gel showed sub-optimal performance due to the irreversible increase in its elastic modulus when exposed to high temperature. All formulations showed excellent physical stability. Comparison of drug release rates revealed that microemulsions yielded significantly higher flux rates (10-11 fold) than conventional ointments. However, the choice of thickening agent played an important role in governing drug release from microemulsions as formulations thickened with Carbopol showed significant decrease in their drug release rate. **Conclusion:** Topical Microemulsions with low surfactant concentrations and improved formulation characteristics, which increased the solubilization and subsequent release of a poorly soluble drug, were developed and their utility demonstrated.

18. In-vitro cytotoxicity of functionalized single walled carbon nanotubes for targeted delivery applications

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Abstract: Carbon nanotubes are a novel class of nanomaterials that have great potential in the field of biomedical research (1). Their ability to enter cells has lead to the exploration of their applications in targeted delivery of various kinds of therapeutic molecules like proteins, nucleic acids, drugs, antibodies and other therapeutics (2). Functionalized carbon nanotubes have recently been shown to be useful in cancer therapy. This study investigates the cytotoxic effects of functionalized single walled carbon nanotubes at different concentrations (SWNTs) on a colon cancer cell line (3,4). Colorectal cancer cells were exposed to single walled carbon nanotubes functionalized with a green fluorescent protein expressing plasmid. The nanotube- plasmid DNA complexes were analyzed by transmission electron microscopy and agarose gel shift assay. The internalization of the nanotube-plasmid DNA complexes was visualized by fluorescence microscopy. The cytotoxicity of the functionalized nanotubes was studied using a tetrazolium based colorimetric assay. Cell count, viability, adherence and cell health were

monitored and evaluated frequently. The results indicate successful functionalization of the nanotubes and subsequent internalization of the nanotube-plasmid DNA complex by the cancer cells. The cytotoxicity of the device was found to be significantly lower compared to a control. Cell viability was shown to have reduced with an increase in carbon nanotube concentration, which means to say that at higher dosages carbon nanotubes could be cytotoxic. Our results show that single walled carbon nanotubes can be successfully used in gene delivery applications and their cytotoxic effects can be limited by optimizing the dosage levels. **References:**

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19. New generation of MUC1 antigens and Toll-like receptor ligands in biodegradable nanoparticles for anticancer immunotherapy

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Purpose: Immunization of antigens and immunomodulators elicits potent cellular immune responses. In the current study, we investigated the influence of subcutaneous vaccination of mice with BC1-042, a TLR ligand, along with a new MUC1 antigen (BC1-244) in the poly (D,L-lactic-co-glycolic acid) (PLGA) nanoparticles on the anticancer immune response in vivo. **Methods:** Biodegradable nanoparticles encapsulating BC1-244 and BC1-042 were prepared using PLGA co-polymer by a double emulsion solvent evaporation method and evaluated with respect to their physicochemical characteristics. The immunogenicity of nanoparticles was assessed after a single or booster immunization in mice with T cell proliferation and IFN-γ ELISPOT in the lymph nodes, and IgG and IgM antibody assay in the blood. **Results:** Immunization of mice with BC1-244 nanoparticles induced potent T cell responses which were furthermore enhanced when BC1-042 was co-delivered in the same vaccine. T lymphocytes obtained from mice immunized with the vaccine formulation showed higher proliferation and cytokine secretion in response to *in vitro* re-stimulation with BC1-244 compared to mice that received only MUC1 antigen. **Conclusion:** The results demonstrates that targeting of BC1-244 and BC1-042 in PLGA nanoparticles is a promising approach for generating potent Th1 polarizing immune responses for the immunotherapy of cancer.

20. Peptide decorated polymeric micelles for selective intracellular drug delivery to metastatic breast cancer cells

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Purpose: The aim of this work is to develop polymeric micellar delivery systems decorated with either P-160 (Figure1), c(RGDfK) or both peptides on their surface and compare the efficacy of polymeric micelles bearing either single or double targeting ligands for active drug targeting to breast cancer cells. **Methods:** Acetal-poly(ethylene oxide)-*b*-poly(α-benzylcarboxylate-ε-caprolactone) (Acetal-PEO-*b*-PBCL) was synthesized by ring opening polymerization of α-benzylcarboxylate-ε-caprolactone using Acetal-PEO as initiator and stannous octoate as catalyst. After micellization, the acetal groups on the surface of polymeric micelles were converted into aldehyde by acidification. Through Schiff base formation, the peptides (P160 and/or c(RGDfK) were conjugated to the aldehyde bearing micelle. ^{1}H NMR and GPC were used to characterize the prepared polymers. The conjugation efficiency of the peptide was determined using gradient reversed phase HPLC. The hydrophobic fluorescent probe, DiI, was physically loaded into the micellar carriers. The cellular binding and uptake of DiI loaded peptide-micelles by MDA-MB-435 human breast cancer cells was studied at 37°C and compared to DiI loaded acetal micelles or free DiI using fluorescence spectroscopy, flow cytometry and confocal microscopy. The uptake of polymeric micelles by MDA-MB-435 cells was also evaluated using a competition experiment in the presence of free peptides under study using fluorescence spectroscopy. **Results**: Single targeted micelles having either P160 or c(RGDfK)

peptides, and double targeted micelles having both peptides on the micellar shell were successfully prepared. An increased uptake for the encapsulated DiI in P160 micelles in comparison to acetal micelle by MDA-MB-435 cells at 370C was revealed by fluorescence spectroscopy and flow cytometry studies. Moreover, the addition of free P160 reduced the uptake of P160 micelles at 37^0C, but didn't affect the cellular association of unmodified micelle, pointing to the involvement of receptor mediated endocytosis in the cell uptake of P-160 micelles. Polymeric micelles decorated with c(RGDfK) also showed higher uptake by MDA-MB-435 cells compared to acetal bearing micelles. Meanwhile, micelles decorated with both P160 and c(RGDfK) at the same time showed slightly higher uptake of encapsulated DiI when compared to micelles decorated with c(RGDfK) alone, but lower uptake than micelles modified with P160 alone. The confocal microscopy images confirmed the internalization of peptide decorated polymeric micelles by MDA-MB-435 cells. **Conclusions**: The results points to a potential of P160 modified polymeric micelles for active drug targeting in breast cancer. The selectivity of polymeric micelles for tumour cells can be enhanced through decoration of micellar surface with multiple ligands for cancer associated receptors. Assessment of the validity of this hypothesis requires further investigations. **Acknowledgments**: Mostafa Shahin is sponsored by a mission from the Egyptian government.

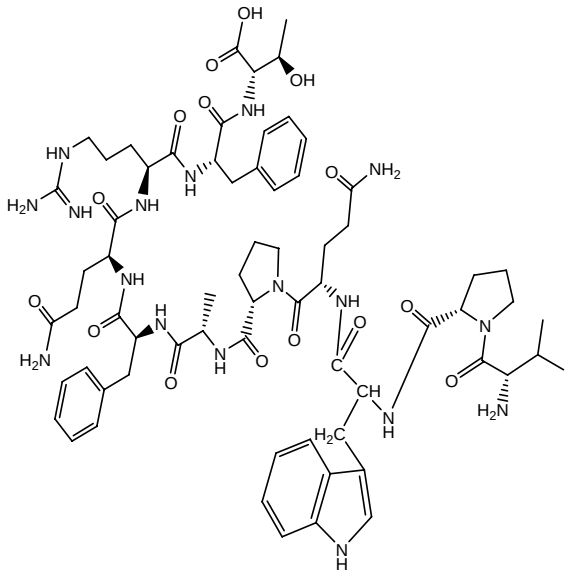

Figure 1: Chemical structure of P160 peptide

21. Chemical tailoring of the hydrophobic block in self associating methoxy poly(ethylene oxide)-*b*-poly(ε-caprolactone) with cholesteryl side chains for the solubilization of STAT-3 inhibitor Cucurbitacin I

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Purpose- Enhanced degree of compatibility between drug and micellar core has been shown to lead to better encapsulation efficiency and lower rate of drug release from these carriers. The aim of this study was to develop methoxy poly(ethylene oxide)-*b*-poly(ε-caprolactone) (MePEO-*b*-PCL) containing cholesteryl side chains on their hydrophobic block, i.e., MePEO-*b*-poly(α-cholesteryl carboxylate ε-caprolactone) (MePEO-*b*-PChCL), and assess the efficacy of cholesteryl containing nanocarriers in improving the solubilization and controlled delivery of chemically compatible drug, cucurbitacin I (CuI). **Methods-** The compatibility between CuI and PCL, PChCL and poly(α-benzylcarboxylate-ε–caprolactone) (PBCL) was assessed calculating Flory-Huggins interaction parameter. MePEO-*b*-PChCL block copolymer was prepared by synthesizing substituted monomer, i.e, α-cholesteryl carboxylate-ε-caprolactone by anionic activation of ε-caprolactone and further ring opening polymerization of substituted monomer using methoxy PEO (5000 $g.mol^{-1}$) as initiator and stannous octoate as catalyst. Synthesized block copolymer was characterized, assembled to polymeric nanocarriers and characterized for their size,

morphology and critical micellar concentration (CMC). Nanocarriers self assembled from MePEO-*b*-PCL, MePEO-*b*-PChCL and MePEO-*b*-PBCL were used to encapsulate CuI. The level of encapsulated CuI in different nano-formulations was measured by LC/MS technique. CuI release from the polymeric nanocarriers was investigated through dialysis of formulations and free CuI at 37 ºC against distilled water. The level of remained CuI in the dialysis bag was measured by LC/MS. **Results-** Synthesis of MePEO-*b*-PChCL was confirmed from ^{1}H NMR. The prepared MePEO-*b*-PChCL block copolymer was able to form true spherical nanocarriers with an average diameter of 195 nm. The predicted compatibility between the drug and different polymeric structures under current study according to the Flory-Huggins interaction parameter (χ_{sp}) were in the order of PChCL>PBCL>PCL. The observed level of CuI encapsulation in different polymeric nanocarriers under the study followed an identical order where cholesteryl containing nanocarrier, i.e., MePEO-*b*-PChCL, exhibited the highest level of CuI loading. However, MePEO-*b*-PChCL based formulation did not show any significant superiority over MePEO-*b*-PCL in controlling CuI release from polymeric nanocarriers. Interestingly, the most efficient control over the rate of CuI release was achieved by benzyl core containing MePEO-*b*-PBCL nanocarriers. **Conclusion-** Engineering of MePEO-*b*-PCL through substitution of cholesteryl side groups on the PCL block may prove to be an efficient strategy for the solubilization of cholesterol compatible drugs such as STAT-3 inhibitor CuI in polymeric nanocarriers.

22. Protective Effect of Scavengers and Surfactants on Gamma Irradiated Cortisone Acetate Aqueous Solutions

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Gamma radiation offers several advantages over other methods as a sterilization technique including the high efficiency, used at room temperature, appreciable penetration to sealed containers and being a terminal and continuous operation. **Purpose:** The purpose of the research is to investigate the protective effect of different types of free radical scavengers or surfactants on cortisone acetate degradation in aqueous solution during sterilization by gamma radiation. **Methods:** Cortisone acetate aqueous solution was subjected to increasing doses of gamma radiation up to the sterilizing dose (25 kGy). The effect of the main radiolytic products of water, Hydroxyl radicals (**OH**$^{\cdot}$), Hydrogen atoms (**H**$^{\cdot}$), and hydrated electrons (**é$_{aq.}$**) was studied separately to identify the most destructive species. **Results:** The results showed a high sensitivity of the drug to radiation even at doses much lower than sterilization. The destructive effects of the radiolytic products of water could be arranged in the following order; **OH$^{\cdot}$> H$^{\cdot}$> é$_{aq.}$** The effect of free radical scavengers, namely methanol and 2-propanol on drug stability to radiation was also investigated. The results revealed a higher protective effect for 2-propanol than methanol. Anionic, cationic or non-ionic surfactants were used in different concentrations below and above their critical micelle concentrations (CMC) to stabilize the drug during irradiation. The presence of cetyl trimethyl ammonium bromide (CTAB), sodium lauryl sulphate (NaLS) or cetomacrogol 1000 as representative examples for cationic, anionic and non-ionic surfactants, showed a protective effect for the drug against radiation. The protective effect was found to be proportional to the surfactant concentration to a certain limit, above which further increase in surfactant concentration had no effect. The maximum protective effect of the ionic surfactants was found to be at their CMC due to the association of the drug with the formed micelles. The stabilizing effect of different surfactants could be arranged in the following order; NaLS > CTAB > cetomacrogol 1000. **Conclusion**: The cortisone acetate aqueous solutions is highly sensitive to gamma radiation even below sterilization dose. The main destructive species of the radiolytic product of water are hydroxyl radicals and hydrogen atoms. Using free radical scavengers such as methanol and 2-propanol have results in a remarkable protective effect which may reach to more than 90% stabilization at radiation dose up to 12 kGy. Different types of surfactants have also shown noticeable stabilizing effect either below or above CMC. The protective effect below CMC is expected to be due to scavenging effect to the surfactant monomers and is proportional to surfactant concentration. While above the CMC, the maximum protective effect could be attributed to the association of the drug with the formed micelle specifically for ionic surfactant.

23. Low-Surfactant Microemulsions for Enhanced Topical Delivery of Poorly Soluble Drugs

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Purpose: To develop low-surfactant microemulsions for enhanced topical delivery of poorly soluble drugs. **Method**: Five different microemulsions were prepared using various oil/surfactant systems. Pseudo-ternary phase diagrams were constructed to obtain the optimum concentration of components, and solubility of a model drug in each formulation was investigated. Three formulations were carried forward for further investigations based on solubilities. Thickening agents were evaluated to increase the skin applicability of the microemulsions. Rheological profiles of each formulation were extensively investigated to evaluate performance criteria such as applicability, processibility, and thermal stability. Formulations were evaluated for physical stability using temperature cycling (5-40°C ramps), accelerated conditions, and centrifugation. *In vitro* release rates of each formulation were determined using Franz diffusion cells. **Results**: The selected microemulsions showed substantially higher solubilizing capacity for the model drug than conventional formulations. Generally, Xanthan gum and Carbopol formulations exhibited excellent and fair rheological properties, respectively. Colloidal silica gel showed sub-optimal performance due to the irreversible increase in its elastic modulus when exposed to high temperature. All formulations showed excellent physical stability. Comparison of drug release rates revealed that microemulsions yielded significantly higher flux rates (10-11 fold) than conventional ointments. However, the choice of thickening agent played an important role in governing drug release from microemulsions as formulations thickened with Carbopol showed significant decrease in their drug release rate. **Conclusion:** Topical Microemulsions with low surfactant concentrations and improved formulation characteristics, which increased the solubilization and subsequent release of a poorly soluble drug, were developed and their utility demonstrated.

24. Synthesis of a novel glucosamine-amino acid derivative and assessment of its stability and gastrointestinal absorption through the rat everted gut

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Purpose: Glucosamine is an amino sugar, which is administered for treatment of osteoarthritis. However, its poor bioavailability (F = 0.19) limits its therapeutic benefits. The ultimate aim of this study is to increase the glucosamine bioavailability. A novel amino-acid derivative of the drug was synthesized. This pro-drug believed to be a substrate for peptide transporter 1 (PEPT-1) and could be easily cleaved into the parent glucosamine in the body after absorption. **Methods**: Using solid phase peptide synthesis method, an ester derivative of the glucosamine and tryptophan (Trp) was synthesized. Briefly, glucosamine was mounted on a resin by its amine group. The carboxyl group of the amino-protected amino acid was activated and bound to the primary hydroxyl group of glucosamine. Eventually the protecting groups were removed and the ester derivative was cleaved from the resin. For assessing the transportability of the pro-drug, everted jejunum sacks were used. Given amount of the ester pro-drug was added to the vessels containing Kreps buffer and everted jejunum sacks. The sacks were oxygenated at 37^{oC} during the study. In another vessel a control test which did not include the gut was conducted. Samples (0.5 mL) were withdrawn from both mucosal and serosal sides at the certain time points and replaced with equal volume of Krebs Buffer. The amount of glucosamine and its ester pro-drug were quantified using HPLC methods. **Results**: The ester was only detectable for few minutes after exposure to the everted jejunum sacks indicating lack of stability. There was no ester pro-drug found in the serosal side indicating its lack of crossing through the gut wall. In the absence of the everted gut, however, the pro-drug exhibited 90% stability during the first hour of incubation. There was approximately 15% of the equivalent initial amount of the pro-drug inside the sack as glucosamine after 1 hour incubation. This indicates cleavage of the pro-drug to glucosamine and subsequent absorption. The extent of glucosamine absorption is in agreement with that observed after incubation of intact glucosamine. **Conclusion:** This ester derivative of glucosamine is stable in Krebs buffer but lacks the desired stability in the gut.

25. Oleic-Acid and Stearic-Acid Modification of Polyethylenimine Improved siRNA Silencing Effect in B16 Melanoma

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Purpose: Oleic acid- and stearic acid-modified derivatives of branched polyethylenimine (PEI) were prepared and evaluated for *in vitro* delivery of small interfering RNA (siRNA) to B16 melanoma cells. **Methods:** Inracellular uptake of siRNA by B16 cells was determined by flow cytometry over 24 hours and was confirmed by confocal microscopy. Modified polymers were compared with commercial transfecting agents to deliver various doses of siRNA to B16 cells. siRNA targeting murine integrin □(v) was used to study the silencing effect mediated by our formulations. **Results:** The formulated complexes were shown to enter B16 cells in a time-dependent fashion, reaching over 90% of the cells after 24 hours, as compared to only 5% of the cells displaying siRNA uptake in the absence of any carrier. Confocal microscopy revealed cytoplasmic, possibly endosomal, and perinuclear localization of siRNA complexes but not naked siRNA. The modified PEIs were superior or comparable to some of the commercially available transfection agents; the hydrophobically-modified polymers gave 3-fold increased siRNA delivery than the parent PEI, ~5-fold higher delivery than jetPEI™ and Metafectene™, a comparable delivery to Lipofectamine 2000™, but a 1.6-fold decreased delivery as compared to INTERFERin™, which was the most efficiency reagent in our hands. Using an siRNA specific for integrin □(v), a dose dependent decrease in integrin □(v) levels was demonstrated by using flow cytometry, which indicated an enhanced reduction of integrin □(v) levels for oleic- and stearic-acid modified PEIs in B16 cells. **Conclusion:** The overall results suggested that the hydrophobically-modified PEIs provide a promising delivery strategy for siRNA therapeutic applications. [1] This work is dedicated to the memory of Dr. John Samuel who passed away during the completion of this study.

26. Carboxymethyldextran-*b*-PEG nanoparticles for aminoglycosides delivery: Preparation and physicochemical characterization

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Purpose: The aim of this study was to prepare nanoparticles by electrostatic interaction between the block copolymer carboxymethyldextran-*b*-PEG (CMD-PEG) and several aminoglycoside antibiotics (neomycin and paromomycin) and to assess their performance as delivery systems. **Methods:** The nanoparticles were characterized by dynamic light scattering (DLS), ^{1}H NMR spectroscopy and scanning electron microscopy (SEM). The antibacterial activity of nanoparticles-loaded antibiotics was tested against pathogenic bacteria, *Escherichia coli* (*E. coli*), *in vitro***.** Isothermal titration calorimetry (ITC) was used to study the binding of neomycin and paromomycin to CMD-PEG at pH 7.0 and 8.0 in four buffers of different heats of ionization. **Results:** The ITC showed that the binding of paromomycin and neomycin to CMD-PEG is accompanied by uptake of protons. The number of protons exchanged is drug and pH dependent. For instance, the binding of paromomycin to CMD-PEG necessitates the uptake of 0.85 ± 0.04 protons at pH 7.0 and 2.03 ± 0.007 at 8.0 whereas for neomycin the uptake is 1.42 ± 0.01 protons at pH 7.0 and 1.71 ± 0.01 protons at pH 8.0. DLS showed that electrostatic interactions between CMD-PEG and either paromomycin or neomycin led to the formation of monodispersed nanoparticles that varied in size from 69 ± 1.39 to 136 ± 3.65 nm and from 74 ± 1.6 to 92 ± 2.5 nm, depending on the amine/carboxylate molar ratio, for paromomycin and neomycin, respectively. ^{1}H NMR spectroscopy indicated that neomycin nanoparticles having an amine/carboxylate molar ratio of 2.0 have a drug content of about 50 % w/w and that the nanoparticles remained intact for up to 2 months. Drug release studies indicated that neomycin was released from the nanoparticles in a sustained manner, where 12 % was released after 24 hours. SEM observations confirmed the formation of spherical, monodispersed nanoparticles. Both free and nanoparticles-loaded drugs have similar activity against *E.*

coli, in vitro. **Conclusion:** This study indicated that the protonation status of aminoglycosides is a critical factor in the formation of nanoparticles based on electrostatic interactions with an oppositely charged polymer. CMD-PEG-based nanoparticles may find applications in the oral or ocular delivery of aminoglycoside antibiotics.

27. Inhalable nanoparticles: a new non-invasive approach for the treatment of lung cancer

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Purpose: The present work explores the efficacy of an effervescent dry powder inhalation formulation containing doxorubicin nanoparticles for the treatment of lung cancer in mice models. **Methods:** Doxorubicin nanoparticles were prepared using n-butylcyanoacrylate monomer. The particle size of the nanoparticles was measured using Zetasizer (Malvern, England). The nanoparticles were incorporated into effervescent carrier particles and the suspension was dried using spray freeze drying technique. For this purpose, nanoparticle suspension was sprayed using a two-fluid nozzle utilizing gaseous nitrogen into a flask containing liquid nitrogen. The liquid nitrogen was allowed to evaporate and form a cake. The vacuum container was attached to a freeze drying system and after 48 hours the powder was collected. The particle size of the powder was analyzed using Mark II Anderson Cascade Impactor. The powder was tested on tumor bearing nude mice (Balb/c) models. The animals were treated once a week for four weeks with either the effervescent doxorubicin nanoparticle powder, equivalent dose of doxorubicin as iv injection or blank inhalable nanoparticle powder. A no-treatment group was included as control in the study. For the delivery of inhalable powder DP-4M device (Penn Century Inc, PA) was used. **Results:** The nanoparticles were in the size range of 130-150 nm. The mass median aerodynamic diameter of the carrier powder was 4.80 ± 0.04 μm which is suitable for lung delivery of the powder. Effervescent doxorubicin nanoparticle powder showed promising results compared to the conventional i.v. injection of doxorubicin. The animals treated with nanoparticle powder of doxorubicin showed longer survival time during the surveillance period. **Conclusion:** This study showed that the effervescent doxorubicin nanoparticle powder is superior to conventional intravenous injection for the treatment of lung cancer. The present inhalable nanoparticles delivered via effervescent carrier particles would thus appear to be a proper route of administration for the treatment of lung cancer.

Natural Products

28. Safety Pharmacology of Cree Medicinal Plants: CYP Inhibition and Cardiac Chronotropic Effects

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Purpose: This study was performed to determine if medicinal plants used by the Cree of Eeyou Istchee (CEI) to treat type 2 diabetes have the potential to cause adverse effects through interactions with drugs by cytochrome P450 (CYP) inhibition, or by altering the chronotropy of neonatal rat cardiomyocytes, *in vitro*.**Methods:** Ethanol extracts from 17 plant species (AD01-AD03, AD06-AD09, AD11, W1-W9) were prepared and examined for their inhibition effect on various CYP members using microtiter fluorometric assays: 2C9, 2C19, 2D6, 2E1, 3A4, 3A5, and 3A7. Four selected species (W2, W4, W5, W9) were also examined for acute chronotropic effects on neonatal rat cardiomyocytes, *in vitro* using micro-electrode arrays (MEAs). **Results:** (a) CYP inhibition - The top three overall high inhibitors were AD01, AD07, and W2. The top three overall lowest inhibitors were AD08, W7, and W9. Comparable results were observed between CYP 3A4, 3A5, and 3A7, which were also the more highly inhibited isoforms. CYP 2C19 had the overall highest inhibition by the 17 plants; whereas, CYP 2D6 had the overall lowest inhibition. (b) Chronotropic effects – None of the extracts tested significantly affected chronotropy; however, the combination of metformin and W9 significantly decreased the chronotropy. **Conclusions:** Overall, several of the extracts (AD01, AD07, W2) highly inhibited most CYPs with the greatest inhibition on CYP 3A and 2C19. CYP inhibition can alter normal drug metabolism and consequently result in adverse drug reactions. W2, W4, W5 and W9 taken alone have no effect on chronotropy, but a concomitant treatment of W9 with metformin can significantly decrease the chronotropy. In conclusion, several of the plants may cause adverse effects through interactions with drugs. Further studies are warranted to determine if these effects are clinically significant.

29. Anti-Cancer Activity, Pre-Clinical Pharmacokinetics, and Stereospecific Separation and Analysis of Sakuranetin in Fruits and Plants

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Purpose: To develop a high-performance liquid chromatographic (HPLC) method to quantify sakuranetin enantiomers; assess its stereospecific pharmacokinetics in rats; racemic *in vitro* anti-cancer activity; and its stereospecific content in different fruits and plants. **Methods:** A novel stereospecific HPLC method was developed using a Chiralpak® AD-RH column, ultraviolet (UV) detection at 288 nm, mobile phase composed of methanol and water (95:5,v/v/v); and flow rate of 0.6 mL/min. Racemic sakuranetin (10 mg/kg) was administered intravenously to male Sprague Dawley rats, collecting serum and urine over 120 h post-dose. Racemic sakuranetin (0-250 µg/mL) was incubated in MDA-MB-231, HCT 116, PC-3, and Hep-G2 (human breast, colorectal, prostate and liver adenocarcinoma cells, respectively). Methanol fractions were extracted from orange, apple, rice and matico (*Piper aduncum* L.) and assessed with and without enzymatic hydrolysis to assess the glycoside and aglycone sakuranetin content. **Results:** Sakuranetin enantiomers were detected in plasma and urine primarily as glucuroconjugates. Similar pharmacokinetic parameters were observed between sakuranetin enantiomers having serum half-lives of 19.57h for R- and 18h for S-sakuranetin; and volumes of distribution (V_d) of ~0.63 L/kg, total clearance (CL_{total}) of 0.024 L/h/kg, and area under the curve (AUC_{inf}) of ~220 µg*h/mL for both enantiomers. Preliminary data shows that racemic sakuranetin has concentration-dependant anti-cancer activity having IC_{50} values in the µM range. Furthermore, S-sakuranetin and S-sakuranetin glycoside were the predominant enantiomer/epimer detected in rice, orange, apple, and matico. **Conclusions:** The stereospecific HPLC assay is sensitive, reproducible, accurate, and specific. Sakuranetin exhibits a large volume of distribution, a long half-life, and undergoes Phase II metabolism as evident by the formation of at least one glucuronide metabolite. Minor differences were observed in the pharmacokinetic parameters between sakuranetin enantiomers. Racemic sakuranetin demonstrates concentration dependent anti-cancer activity. Predominant content of S-sakuranetin and its corresponding glycoside was evident in fruits and plants.

30. Modulation of lipid accumulation in 3T3-L1 cells by selected polyphenols and *Malus x domestica* extracts in an *in vitro* adipogenesis model

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Purpose: To develop an *in vitro* model of adipogenesis using 3T3-L1 pre-adipocyte cells and to assess the ability of selected aglycone polyphenols (naringenin, hesperetin, eriodictyol, phloretin, pterostilbene, and homoeriodictyol), glycosides (naringin, hesperidin, eriocitrin, and phloridzin), and selected apple extracts to inhibit adipogenesis. 3T3-L1 cells are fibroblasts that differentiate into adipose-like cells constituting a useful tool for studying adipogenesis and obesity. **Methods:** The adipogenesis assay kit was purchased through Cayman Chemical. 3T3-L1 cells were maintained in DMEM with 10% FBS. 96 well plates were seeded at 3 x 10^4 cells/well and allowed to grow to confluency. Methanolic extracts of both peel and flesh were taken from red 'Delicious' and 'Gala' apples. Two days post-confluency, the media was changed to an induction medium containing IMBX, insulin, dexamethasone, and treatment compound (1-250 µg/ml) or extract (0.2-20 mg/ml). At five and then seven days post-confluency, the media was changed to media containing insulin and treatment compound (1-250 µg/ml) or extract (0.2-20 mg/ml). Nine days after confluency, lipid accumulation in the cells was stained with Oil Red O by the method provided with the assay kit and absorbance of each well read at 492 nm. **Results:** All selected polyphenol aglycones had greater ability to inhibit differentiation than their corresponding glycosides. IC_{50} values for inhibition for the aglycones including naringenin, hesperetin, eriodictyol, phloretin, pterostilbene, and homoeriodictyol were 92.6, 53.7, 53.5, 73.2, 23.9, and 73.4 µg/ml, respectively. IC_{50} values for the glycosides

could not be determined due to limited activity. 'Delicious' peel and flesh extracts exhibited greater inhibition than peel and flesh extracts from 'Gala' apple. **Conclusions:** The sugar moiety present on the glycosidic forms of polyphenols appears to hinder the anti-adipogenic pharmacological effect. Liberation of the aglycone form after oral consumption may allow inhibition of adipogenesis and therefore may be useful in the prevention of obesity.

31. Examination of selected polyphenols and *Malus x domestica* extracts in attenuating inflammatory bowel disease in *in vitro* and *in vivo* models

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Purpose: To assess anti-inflammatory activity of selected polyphenols and apple extracts to attenuate inflammatory bowel disease (IBD) in an *in vitro* model using HT-29 cells and an *in vivo* rat model. **Methods:** For the *in vitro* model, HT-29 cells were treated with polyphenol aglycones (phloretin, pterostilbene, hesperetin, naringenin, eriodictyol, homoeriodictyol, and taxifolin) (1-250 µg/mL), glycosides (hesperidin, naringin, and eriocitrin) (1-250 µg/mL), or methanolic peel or flesh apple extracts (0.2-20 mg/ml). Inflammation was induced with TNF-α and prostaglandin E_2 (PGE_2) release in media was measured using a commercially available ELISA kit. For the *in vivo* model, male Sprague-Dawley rats were administered either vehicle, dexamethasone (positive control), or polyphenol (10 mg/kg for phloretin, pterostilbene, resveratrol, rhapontigenin, and piceatannol; 20 mg/kg for hesperetin, hesperidin, naringenin, and naringin), via oral gavage at 48h, 24h, and 1h pre-induction and 24h post-induction. Colitis was induced via rectal administration of trinitrobenzene sulfonic acid (TNBS). One hour following final treatment, 1 g of phenol red was administered via oral gavage. Urine was collected 24h later and absorbance measured at 559 nm. Dissected colons were macroscopically evaluated assessing for the presence of diarrhea, ulcers, and inflammation. **Results:** All the polyphenol aglycones, glycosides and apple extracts exhibited a concentration-dependant reduction in PGE_2 levels in the i*n vitro* model. Red 'Delicious' apple extracts showed greater activity than 'Gala' extracts. All assessed polyphenols attenuated the effects of IBD *in vivo* as indicated by both colon macroscopic scoring and quantification of phenol red in urine, some polyphenols exhibited greater activity than dexamethasone. **Conclusions:** The effects of polyphenols in the *in vitro* model paralleled the *in vivo* model. The examined polyphenols/extracts exhibited activity in attenuating the effects of IBD in both *in vitro* and *in vivo* models indicating that consumption of these polyphenols in the diet may help in the prevention/treatment of IBD.

32. Effect of African potato (*hypoxis hemerocallidea*) on the pharmacokinetics of efavirenz in healthy male volunteers

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Purpose: The use of natural health products (NHPs) as a traditional medicine (TM) among people living with HIV/AIDS is widespread, although the effects of these medicines on the pharmacokinetics of antiretroviral medicines (ARVs) have not yet been established. The purpose of this study was to evaluate the effect of African Potato (AP) on the pharmacokinetics of efavirenz (EFV). **Methods:** A single dose, two-phase sequential study was conducted over a period of 31 days in 10 healthy volunteers. On day 1 of the study volunteers were administered a 600 mg EFV tablet and blood samples were collected before dosing and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 12.0, 18.0, 24.0, 36.0 and 48 hrs post dosing. From day 16, a traditionally prepared AP decoction was administered daily until day 30. On day 29, volunteers were administered a single 600 mg dose of EFV as was done on day 1. Plasma samples were harvested immediately after blood sample collection and frozen at -80 ºC until assayed. Plasma concentrations of EFV were determined by a validated HPLC method with UV detection and pharmacokinetic parameters were calculated. Geometric mean ratios of C_{max} and $AUC_{0\text{-}48}$ of EFV before and after co-administration of 14 successive daily doses of AP were compared. **Results:** All subjects completed the study

and the geometric mean ratios of Cmax and AUC_{0-48} were 97.30 and 102.82 with corresponding 90% confidence intervals (CIs) of 78.81-120.14 and 89.04-118.80, respectively. **Conclusions:** Pharmacokinetic data generated during this study indicated that African Potato did not significantly alter the pharmacokinetics of EFV. Hence, co-administration of AP is unlikely to affect the clinical use of EFV. **Acknowledgements:** Financial support from the Ontario HIV/AIDS Network (OHTN) is gratefully acknowledged

Clinical Sciences

33. Prospective Study of the Prevalence and Treatment of Major Depression in AL-RAZI Hospital, Tripoli-Libya

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Purpose: The main objective was to study the prevalence of major depression in inpatient admitted to Al-Razi hospital for neurology and psychiatric disorder in Tripoli – Libya during the year (2005) and to assess treatment strategies in the outpatient clinic. **Method:** 100 cases were selected in a random manner out of 1729 inpatients hospitalized during the year 2005 with depressed mood. Data extracted were obtained from the inpatient files. The selected patients were then followed up for one year in the out-patients department (OPD) to find out the type and the outcome of the given treatments. **Results and discussion:** In the selected 100 patients 48% had major depression and the rest had other different types of mental illnesses mixed with depression. 98% of the selected cases were males and only 11% were females. The lower percentage of the female patients might reflect the social stigma induced by psychiatric illnesses and the avoidance of the Libyan family to hospitalize female patients. 16% of the patients had suicidal tendency which is in the same range reported globally. Mostly half of the depressed patients (56%) were between 15 and 30 years old; where 42% of these patients (mostly male) were addict to certain drug(s) like marijuana, tobacco and alcohol. We found that 98% of the patients were treated with the first generation (typical) antipsychotic drugs (FGAs) and 48% were given the conventional tricyclic antidepressants. After follow-ups in OPD we found that 54% of the patients who took their medication properly and regularly responded positively. On the other hand, the percentage of regular follow up after discharge from the hospital was only 19% and the percentage of relapse after successful treatment was found to be around 30%. These poor follow-ups could be related to many reasons including, the patients consulting with other specialized hospitals outside the country (like Tunisia), or that patients seeked other forms of therapy from non-specialized people. The high percentage of relapse may reflect patients' incompliance with the therapy or due to the changes in drug formulation due to changes of drug suppliers to Al-Razi hospital. **N.B**. This research was presented as a poster in the Eleventh Scientific Meeting of the Association of Colleges of Pharmacy in the Arab World held in Tripoli Dec. 2007, but was not published in any scientific journal.

34. Characterizing use of acetylsalicylic acid within a long-term care facility

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Purpose: This cross-sectional study was designed to describe acetylsalicylic acid (ASA) use for cardiovascular risk reduction in elderly residents of a long-term care facility. The specific objectives were to identify the proportion of residents with: 1) any indications for ASA use; 2) any contraindications to ASA use; and, 3) ongoing ASA use. **Methods**: Eligible subjects lived at a privately owned long-term care facility and received their medications from a single community pharmacy. Facility charts and medication profiles were reviewed to identify indications and contraindications for ASA use. Indications for ASA use were defined by current clinical practice guidelines for diabetes, heart failure, post-myocardial infarction, peripheral vascular disease, and stroke. Contraindications were identified from the product monograph in Access Medicine. **Results**: We reviewed the charts for 92 residents (18 male (20%); mean age 83 ± 9.5 years) and found that all 92 (100%) had an indication for ASA to reduce the risk of cardiovascular disease. Of these 92 individuals, 41 had no contraindications for ASA use. Of the 41 residents who could be using ASA, 21 (51%) were taking ASA regularly, 8 (20%) used an alternative antiplatelet or anti-thrombotic agent, and 12 (29%) were not using any agents. **Conclusion**: Compared

to other groups where ASA use to lower cardiovascular risk is approximately 34%, residents in this facility were more likely to be using ASA or an alternative antiplatelet or anti-thrombotic agent. However, 29% of these residents were not using any antiplatelet or anti-thrombotic agent despite having an indication and no contraindications.

Pharmaceutical Chemistry

35. Lanthanide containing compounds for the treatment of bone density disorders

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Purpose. Lanthanide ions, Ln(III), are known functional mimics of Ca(II) ions and have been shown to affect the bone remodeling cycle. Exploiting this disruption to the bone remodeling cycle has potential for the treatment of bone density disorders, such as osteoporosis. Lanthanum carbonate has been proposed as a potential preventative measure for post-menopausal osteoporosis; however, known negative side effects (e.g. gastrointestinal upset) have obviated its development. In an effort to find new orally available active agents for bone density disorders that circumvent the problems associated with lanthanum carbonate, our group is investigating a series of lanthanide containing complexes incorporating small, non-toxic, bidentate pyrone and pyridinone ligands. These ligands increase the water solubility and bioavailability of the lanthanide ions, hopefully diminishing gastrointestinal side-effects **Methods.** Bidentate pyrone and pyridinone neutral lanthanide complexes have been synthesized (LnL_3, Ln = La, Eu, Gd, Tb, Yb, L = 3-hydroxy-2-methyl-4-pyrone (ma^-), 3-hydroxy-2-ethyl-4-pyrone (ema^-), 3-hydroxy-1,2-dimethyl-4-pyridinone (dpp^-) and 3-hydroxy-2-methyl-4(1H)-pyridinone (mpp^-)) and fully characterized by IR, UV-visible, EA and NMR spectroscopy. Non-cytotoxic concentration of LnL_3 (determined using mitochondrial respiration as a marker) were added to Caco-2 cells and their cell uptake and directional flux of the complexes were determined by ICP-MS methods. I*n vitro* hydroxyapatite (HA) binding studies were also completed on the lanthanide complexes. The proportion of intact compounds bound to HA was calculated based on determination of Ln(III) concentration by ICP-MS and by UV-vis spectrophotometric assay of the proligand in solution. **Results.** The LnL_3 species were found to have IC_{50} values at least 6 times greater than that of cisplatin, $\geq$ 98% HA-binding capacity, and permeability coefficients in the moderate range, similar to Fosrenol™, an FDA approved lanthanum-containing phosphate binder. In addition, the LnL_3 complexes did not appear to disturb the HA structure upon binding. $La(dpp)_3$ has ascertained to be the lead compound for the treatment of bone density disorders with the highest percentage cell uptake of 9.07 $\pm$ 2.33% and the highest preliminary P_{app} value of 3.54 $\pm$ 2.86 x 10^{-6} cm s^{-1} compared to the other LnL_3 complexes tested. **Conclusions.** Twenty LnL_3 were successfully synthesized using Hma, Hema, Hmpp, or Hdpp as proligands and their potential as a therapeutic drug for bone resorption disorders were assessed. These novel LnL_3 thus have potential as orally available bone resorption inhibition drugs, with minimized side effects that may also serve as preventative supplements for osteoporosis. **Acknowledgements.** This work was previously presented at Dalton Discussion No. 10, September 3-5, 2007, University of Durham, Durham, UK and published in Dalton Trans., 2007, 5019-5039.

36. Interaction between class IIa bacteriocins and a bacterial cell membrane model: A molecular dynamics simulation study

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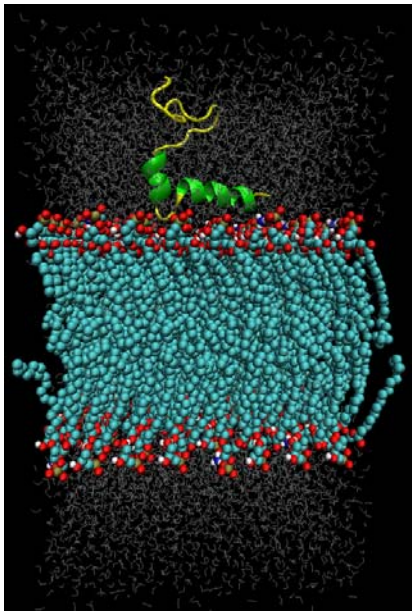
Purpose: The aim of this study is to investigate the equilibrium positions and interaction of four different antimicrobial peptides of class II bacteriocins with a bacterial cell membrane model by molecular dynamics (MD) simulation. **Methods:** MD simulations were generated by placing Carnobacteriocin B2 (CbnB2), Curvacin A Leucocin A and Pediocin PA at the interface of a Gram positive cell membrane model consisting of 96 POPG and 32 POPC molecules homogenously distributed in a bilayer structure and placed in 6 x 6 x 10 nm cuboid box. The box was solvated with SPC water molecules. Na and Cl ions were added to make the box electroneutral. Two more

simulations were generated by placing CbnB2 in the bilayer at two different orientations. MD simulations were then run for 50 ns. **Results:** Over 0.3 μs of MD simulations have been done to investigate the different aspects of interactions of class II bacteriocins with bacterial cell membrane. Fairly strong electrostatic interaction developed between the positively charged peptides and the negatively charged lipid bilayer. Our results indicate that these bacteriocins are well equilibrated at the lipid/water interface. 15-20 hydrogen bonds between the bacteriocin and the lipid head groups were observed after five ns maintained and maintained till the end of simulation times. Peptides secondary structures were conserved all over the simulations. **Conclusions:** Bacteriocins are attracted and strongly held at the lipid/water interface. Their secondary structures were stabilized at the interface with electrostatic interaction and H-bonding between the bacteriocins and the bilayer. The results from these simulations could point out the key elements necessary for the antimicrobial effect of such bacteriocins and pave the way for better understanding of the mechanism of action of these potent antimicrobial peptides which ultimately lead to better designing of new antimicrobial agents.



Poster Presentations

Saturday, May 24, 2008

37. Arachidonic acid metabolism in isoproterenol-induced cardiac hypertrophy model of Sprague Dawley rat

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Purpose: Cardiac hypertrophy is one of the early complications leading to the development of heart failure and consequently heart death. The role of cytochrome P450 (CYP) in cardiovascular health and disease is well established. Many CYP enzymes have been identified in the heart and their levels have been reported to be altered during cardiac hypertrophy. Moreover, there is a strong correlation between CYP-mediated endogenous metabolites and the pathogenesis of cardiac hypertrophy. Therefore, we investigated the effect of cardiac hypertrophy on arachidonic acid metabolism and the CYP enzymes responsible for their metabolism. **Methods:** Cardiac hypertrophy was induced by daily intra-peritoneal injections of 5 mg/kg isoproterenol for seven days. Animals were euthanized 24 hours after the last injection. Thereafter, the heart was harvested and the total tissue microsomal protein was isolated. The expression of hypertrophic marker, atrial natriuretic peptide (ANP) and different CYP genes were determined by Western blot analysis. Heart microsomal protein from control or hypertrophied rats was incubated with 50 μM arachidonic acid and arachidonic acid metabolites were determined using LC-MS system with electrospray ionization source. **Results:** Our results clearly demonstrated that isoproterenol-induced cardiac hypertrophy significantly increased the heart weight to body weight ratio as well as the hypertrophic marker, ANP. Incubation of microsomal protein with arachidonic acid resulted in formation of 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acids (EETs), their corresponding dihydroxyeicosatrienoic acids (DHETs), and 20-hydroxyeicosatetraenoic acid (20-HETE). On the other hand, isoproterenol-induced cardiac hypertrophy significantly reduced 5,6-, 8,9-, 11,12-, and 14,15-EET formation and significantly increased their corresponding 5,6-, 8,9-, 11,12-, and 14,15-DHET and 20-HETE. The changes in EETs and 20-HETE formation were associated with a decrease in EETs forming enzymes; CYP2C23 and CYP2J3, and increase in 20-HETE forming enzymes; CYP1B1 and CYP2C11 (form both EETs and 20-HETE) protein expression. **Conclusion:** Cardiac hypertrophy alters arachidonic acid metabolism and their associated CYP enzymes, suggesting their role in development of cardiac hypertrophy. **Acknowledgements:** This work was supported by a Grant from the Heart and Stroke Foundation of Alberta, NWT, and Nunavut to A.O.S.E. M.E.A. is the recipient of Egyptian Government Scholarship.

38. Pharmacokinetics of cladribine and chitosan encapsulated cladribine in rats and zebrafish following a single dose

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Purpose: To study the pharmacokinetics and targeting of cladribine in plasma using a rat model and zebrafish model. **Methods:** Male SD rats (Charles River Laboratories, n = 6) and zebrafish weighing between 300 - 450 g and approximately 1 g (n = 3 at each sample time), respectively were used. Each rat received cladribine (Sigma-Aldrich Chem) 2 mg/kg subcutanously (sc). Each zebrafish received cladribine (2 mg/kg) or chitosan encapsulated cladribine (0.5 mg/kg) by peritoneal (ip) injection. Plasma samples were collected at 0, 15, and every 30 min for 3 – 4 h after injection. Plasma concentrations of cladribine were determined by an established HPLC. Pharmacokinetic data were calculated by standard procedures using the mean data assuming a 2-compartment open model following iv bolus using WinNonLin (Ver 5.01, Pharsight Corp., Cary, North Carolina, USA) and Rstrip (Ver 5, MicroMath Inc., Saint Louis, MO, USA). **Results:** Plasma concentrations of cladribine decreased rapidly following a biphasic decline after sc administrations of un-encapsulated cladribine**,** but appeared to decline monoexponentially after injection with the chitosan encapsulated cladribine. The CL of cladribine were 15.2, 1.2 and 11.3 mL/min/kg in rat, zebrafish, and

zebrafish treated with chitosan encapsulated cladrine, respectively. The t½ (α and β) were 0.5 and 22 hr, 0.2 and 28 hr and 0.9 hr, and the Vdss were 19, 2.8 and 0.9 L/kg respectively in these preclinical models. **Conclusion**: Zebrafish have potential as a preclinical model for pharmacokinetic studies of nucleoside anti-cancer drugs. Chitosan encapsulation increased clearance and reduced Vdss of cladribine in the zebrafish model (Supported in part by a Nova Scotia Health Research Foundation Innovation Grant).

39. Plasma concentrations of adenosine and its oxypurine metabolites in rats following multiple doses of dipyridamole

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Purpose: To study the effect of dipyridamole on plasma concentrations of adenosine and its oxypurine metabolites using an experimental rat model *in vivo*. **Methods:** Male sprague-dawling rats (n= 6) were purchased from Charles River Laboratories and kept in the Carleton Animal Care Center with food and water *ad libitum* for one week prior to experiment. An indwelling catheter was implanted into the left carotid artery of each rat for collecting blood samples. Each rat was given dipyridamole by subcutaneous injection (5 mg/kg) BID for 5 doses. Serial blood samples were taken from each rat for 6 hours following the last injection. A "Stopping Solution" is immediately added to all blood samples and each was centrifuged to separate plasma from red blood cells. Plasma samples were kept at –80^{o}C until analysis by a previously described HPLC method. **Results**: In healthy rats, the concentration of adenosine and all oxypurine metabolites were measurable in plasma. However, plasma concentrations of adenosine, inosine and guanosine in rats treated with dipyridamole were not detectable, and that plasma concentrations of uric acid were only measurable in some samples. The average plasma concentrations of hypoxanthine and uric acid in dipyridamole treated rats were 26.6 ± 4.3 and 1.6 ± 0.7 uM, which were significantly lower than the concentrations found in control rats (52.1 ± 8.4 and 25.9 ± 27.3 uM, respectively) ($p < 0.05$). **Conclusion**: Dipyridamole alters metabolism of adenosine and its oxypurine metabolites in rats *in vivo*. (Supported financially in part by a Rx&D/CIHR summer research studentship and a CIHR-NSHRF-PEF Nova Scotia Regional Partnership Grant.)

40. The battle continues: expression of selected genes in human colon cancer

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Purpose: Most of the patients diagnosed of colon cancer, the third most common cancer in Canada, are 70 years or older. However, the treatment of colon cancer remains less successful mainly due to the lack of effective chemotherapeutic drugs and drug intolerance in the elderly patients. In this study, we illustrated that strengthening the cell protection functions inside the cancer cells, widely used in traditional Chinese medicine, is a valid alternative of the method of destroying the cancer cells directly, which is usually associated with higher cellular toxicities. **Methods:** The expression pattern of a group of ten genes, which were structurally and functionally unrelated, was studied quantitatively by the real-time RT-PCR method in the different clinical stages of human colon cancer using an Origene colon cancer qPCR array panel. **Results:** The expression of the phosphoinositide-3-kinase gene, which controls important cellular activities like proliferation, differentiation and survival, remained unchanged in colon cancer patients, implying that the basic cellular activities were not disturbed inside the cancer cells. However, the expression levels of both the tumor suppressor genes *pten* and *riz1* and the proto-oncogenes *pim3* and *riz2* were elevated, suggesting the battle between the *Yin* and *Yang* sides of tumor genesis continued even in the cancer cells. **Conclusion:** This study supports the concept of *Yin-Yang* regulation of cancer cell development and that chemical agents that can strengthen the force of the *Yang* side (*i.e.* cellular protection) are equally effective as those traditional "silver bullet" drugs targeting the *Yin* side (*i.e.* tumorigenesis) in fighting colon cancer. **Acknowledgements:** This work was supported by a research grant from the Cancer Research Society Inc. and a Research Trust grant from the College of Pharmacy and Nutrition, University of Saskatchewan.

41. In vitro evaluation of glucosamine transport through the everted rat gut

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Purpose: Glucosamine is an amino monosaccharide that is used for the treatment of osteoarthritis. Our previous work has suggested an absolute oral bioavailability of about 19%. The gut, and not the liver, is responsible for its low bioavailability. It has been suggested that the oral absorption of glucosamine is, at least in part, facilitated by the low affinity glucose transporter. The objectives of this study were to determine the mechanism behind this observation, and identify if glucose influences glucosamine oral bioavailability. **Methods:** Everted segments (n=15-20/group) of the rat gut were incubated in oxygenated Krebs Henseleit buffer at 37^{o}C containing 20, 40 and 80 μg/mL glucosamine, respectively. Samples (0.5 mL) from both serosal and mucosal sides were collected at different time intervals up to 150 min and analyzed for glucosamine using HPLC. The mass balance of what lost and gain was determined. The experiment was repeated in the presence of 0, 10 and100 mmoL glucose to examine the effect of glucose level on the gut availability of glucosamine. **Results:** Glucosamine was stable in the presence of everted gut. The decrease in glucosamine concentration in the mucosal side was accompanied by corresponding gain to the serosal side. About 24% of the dug was absorbed in 150 min, which is almost in line with the previous in vivo results. No significant difference was observed in the percent absorbed with 20 (23.78 $\pm$ 11.64%) and 40 (24.01 $\pm$ 19.53%) μg/mL. The extent of absorption after incubation with 80μg/mL, however, was significantly reduced (11.97 $\pm$ 8.61%). Glucose had no significant effect on the glucosamine gut permeation. **Conclusion**: The low oral bioavailability of glucosamine may be attributed to the saturation of the transporters involved in its absorption and not to its instability in the gut. Glucose does not compete with glucosamine absorption through everted gut. (A.I. is a recipient of a scholarship from the government of Egypt).

42. Overexpression of CYP2J2 Provides Protection against Doxorubicin Induced Cardiotoxicity

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Background: It is well known that cardiotoxicity is a major adverse effect caused by doxorubicin, which is a potent anticancer agent. Human CYP2J2 gene is abundant in heart and active in the biosynthesis of epoxyeicosatrienoic acids (EETs), which can protect the myocardium from cardiac injury. Transgenic (Tr) mice with cardiomyocyte-specific overexpression of human CYP2J2 gene had enhanced postischemic functional recovery of left ventricular function. However, whether the overexpression of CYP2J2 can play the functional role against doxorubicin-induced cardiotoxicity remains unknown. **Purpose:** This study was undertaken to test the hypothesis that the overexpression of CYP2J2 can provide protection against doxorubicin-induced cardiotoxicity. **Methods:** Tr mice with cardiomyocyte-specific overexpression of human CYP2J2 gene and their wild-type (WT) littermates were treated with doxorubicin in acute (4d; 3x 0, 5 or 15mg/kg) and chronic (7wk; 10x 0, 1.5 or 3.0mg/kg) protocols. **Results:** Acute treatment resulted in marked elevation of serum lactate dehydrogenase and creatine kinase levels that were significantly higher in WT than CYP2J2 Tr mice. Increased activity of stress induced enzymes was significantly larger in WT than CYP2J2 Tr mice (catalase: 750 vs. 300%; caspase-3: 235 vs. 165%). Following chronic treatment, comparable decreases in body weight were observed in both WT and CYP2J2 Tr mice. Cardiac output assessed by measurements of fractional shortening using M-mode transthoracic echocardiography showed CYP2J2 Tr and WT mice had comparable basal contractile function (WT 50±2; CYP2J2Tr 50±1%). However, following chronic doxorubicin treatment CYP2J2 Tr mice had significantly better cardiac output (WT 37±2%; CYP2J2Tr 47±1%). Interestingly, a decreased ratio of β-myosin heavy chain (βMHC):α-myosin heavy chain (αMHC) was observed in Tr mice compared with WT. **Conclusion:** Together, these data suggest that the overexpression of CYP2J2 are cardioprotective against toxic injuries induced by doxorubicin. *Portions of this work were presented at the Cardiovascular Research Group Retreat, June 1, 2007, University of Alberta, Edmonton, AB

43. Effects of lipopolysaccharide on nutrient transporter expression in the human mammary gland epithelial cell line, MCF12A

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Purpose: Generally, maternal bacterial infections are not contraindications to breastfeeding. However, little research addresses the potential adverse consequences associated with maternal inflammatory diseases and nutrient transporter interactions at the lactating mammary gland. The purpose of this research was to investigate the effects of a gram-negative bacterial stimulus, lipopolysaccharide (LPS), on nutrient transporter expression in mammary epithelial cells. **Methods:** The human mammary gland epithelial cell line, MCF12A, was incubated with 1μg/mL LPS for 6 hours. mRNA expression levels of the cytokines, tumor necrosis factor-α (TNF- α) and interleukin-6 (IL-6), as well as concentrative nucleoside transporters (CNTs), organic cation/carnitine transporters (OCTNs), facilitated glucose transporters (GLUTs) and sodium dependent glucose transporter 1(SGLT1) were measured using Quantitative RT-PCR. **Results:** LPS administration significantly upregulated TNF-α and IL-6 mRNA levels , indicating an innate immune response of MCF12A cells to LPS. CNT2 and CNT3 expression levels significantly decreased with LPS ($P<0.05$) with a slight decrease for CNT1 ($P>0.05$). Both OCTN1 and OCTN2 mRNA levels showed significant decreases upon LPS exposure ($P<0.05$). Interestingly, mRNA expression of glucose transporters (GLUT1, GLUT8, SGLT1) was significantly increased ($P<0.05$). **Conclusions:** LPS exposure caused significant alterations in nutrient transporter mRNA levels in mammary epithelial cells. Such changes may correspond to altered nutrient levels in breast milk and this warrants investigation. Furthermore, concurrent enhancement in glucose transporter expression with downregulation of the L-carnitine transporters (OCTN) may suggest a switch in metabolic fuel utilization (i.e. glucose in favour of fatty acids) in mammary epithelial cells during inflammatory states. Future studies plan to investigate these observations.

44. Comparative Modeling of Human b-Tubulin Isotypes and Implications for Drug Binding

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Purpose: The protein, β-tubulin is the target for a number of anti-mitotic compounds that bind to and inhibit microtubule dynamics. The existence of several isotypes of β-tubulin, coupled with their varied distribution in both normal and cancerous cells has provided us with a platform upon which to construct novel chemotherapeutic agents that are able to differentiate between these cells. A drug that specifically targets those tubulin isotypes expressed in cancer cells would maintain its cytotoxic activity on these cells, yet have a reduced effect on cells found in normal tissues. To test this hypothesis, we have performed homology modeling on a consensus set of nine human β-tubulin isotypes and analyzed them for differences in the colchicine-binding site. **Methods:** Using the structure of βI tubulin as a template, we have created homology models of the βII and βIII tubulin isotypes. Using the colchcine-binding cavity in tubulin as a template, we have designed several novel colchicine derivatives, which we computationally probed for binding affinity to each of these isotypes using docking and molecular mechanics calculations. Once a set of diverse derivatives had been identified, a small subset was synthesized and tested against a number of cancerous cell lines. **Results**: Changes in the shapes of the binding sites were obvious following relaxation molecular dynamics of the models. There were three chemical differences within the side chains contained in the colchicine binding site that are found within 6 Å of the bound colchicine molecule. Modifications to the methoxy groups in colchicine were synthesized to probe these differences. Cytotoxicity screening was performed on a number of cancerous cell lines based on the cancer of origin and differing morphologies. Cytotoxicity assays were performed, yielding a correlation to computational binding predictions. **Conclusions**: When we compared in vivo cytotoxicity results to predicted binding energies, we obtained a significant correlation. This observation suggests that our modeling of the colchcine binding site will be effective for designing derivatives that are capable of differentiating between tubulin isotypes.

45. Cytochrome P450 (CYP) Metabolites of Arachidonic Acid Influence Adenylate Cyclase Activity

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Arachidonic acid (AA) can be released by phospholipases in response to several stimuli such as ischemia. Cytochrome P450 epoxygenases metabolize AA to epoxyeicosatrienoic acids (EETs) which act as potent signaling molecules in both cardiac and extracardiac tissues. **Purpose:** To investigate the role of EETs in myocardial function and protection against ischemic injury, we developed transgenic mice with cardiac-specific overexpression of CYP2J2 and enhanced cardiomyocyte EET biosynthesis. This study examines the role of cAMP in improved postischemic functional recovery in CYP2J2 transgenic (Tr) mice. **Methods:** Using a Langendorff isolated heart model, we previously showed that postischemic recovery of left ventricular developed pressure (LVDP) was significantly better in CYP2J2 Tr hearts than in wild type hearts (CYP2J2 Tr 37±3%; WT 23±2%, n=26, $p<0.05$). **Results:** This improved functional recovery was associated with higher cAMP levels in CYP2J2 Tr heart tissue following ischemia (preischemic: WT 1080±83; CYP2J2 Tr 1130±143; postischemic: WT 483±36; CYP2J2 Tr 963±51 pmol/g tissue; n=8-16, $p<0.05$). Importantly, perfusion with the selective P450 epoxygenase inhibitor N-methylsulphonyl-6-(2-proparglyloxyphenyl) hexanamide (MS-PPOH, 50μM) for 20min prior to ischemia abolished both the improved postischemic LVDP recovery and elevated cAMP levels in CYP2J2 Tr hearts. Mechanistic studies investigating the role of EETs toward altered cAMP production were carried out in both rat cardiomyocytes and HEK293 cells. Cells treated with 14,15-EET (0, 1 or 10μM) resulted in a dose-dependent increase in cAMP production (2.1±0.6, 4.2±1 and 34±20 pmol cAMP/500,000 HEK293 cells, respectively). Interestingly, co-administration of 14,15-EET (1μM) with forskolin (50μM) attenuated forskolin induced cAMP production (EET/Forsk: 16.7±7; Forsk: 146±33 pmol cAMP/500,000 HEK293 cells). **Conclusion:** Together, these data suggest that improved postischemic function recovery in CYP2J2 Tr mice involves enhanced cAMP production and that EET-mediated signaling involves altered G-protein coupling.

46. Calculation of Normalized Drug Levels in the Presence of Altered Plasma Protein Binding

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Purpose: The plasma protein binding of drugs has been shown to have significant effects on the quantitative relationship between clinical pharmacokinetics and pharmacodynamics. In many clinical situations, measurement of the total drug concentration does not provide the needed information concerning the unbound fraction of drug in plasma which is available for pharmacodynamic action. Therefore, the accurate determination of unbound plasma drug concentrations is important in understanding drug action. However, due to financial constraints or lack of suitable methods, the unbound drug concentration is rarely determined. Instead, a "normalized concentration" is often calculated based on the observed total drug concentration and serum albumin concentration. The method is currently applied for drugs such as phenytoin and warfarin. The scientific literature and therapeutics textbooks list several equations for calculating normalized drug levels, and these equations may lead to different results. Regrettably, all the equations in the current literature are based on an outdated drug binding model to human serum albumin – this important protein is considered to have either a single binding site, or multiple binding sites with identical affinity. Furthermore, the published calculations are based on the fraction of unbound drug, which is known to depend on both protein and drug concentration. On the other hand, recent advances in ligand-receptor binding studies have demonstrated that drug molecules may bind to several binding sites with different affinity (each site on albumin has its own distinct binding constant). In response to the new scientific evidence about drug biding to human serum albumin, the purpose of the present study is to develop a general method for calculating normalized drug levels in the presence of altered plasma protein binding. **Methods**: When several molecules of drug can be bound by a protein molecule, multiple equilibria are established; these equilibria may be formulated in terms of a stoichiometric analysis or on the basis of a site-oriented scrutiny. The stoichiometric model is based on stepwise equilibria and binding is expressed as a function of stoichiometric binding constants (k_i); this model accommodates interactions between sites that accentuate or attenuate binding affinities. In the case of the site-oriented approach, each site is considered to have a fixed, invariant affinity, and its binding of ligand is independent of events at other sites. Accordingly, stoichiometric binding constants (k_i) are replaced with site binding constants

(K_i). Both binding models are currently encountered in the scientific literature; hence, drug binding constants may be reported either as k_i or K_i. The binding constants derived by the two approaches are not the same in magnitude or in the binding step to which they must be assigned. Nevertheless, the present study shows that the normalized drug concentration (C_{norm}) may be expressed for both binding models as

$$C_{norm} = C_{obs} \cdot R - z_1 \cdot (R - 1) \quad (1)$$

where C_{obs} is the observed drug concentration, R is the ratio between the normal protein concentration and observed protein concentration, and z_1 is a parameter derived from the binding model. **Results:** The proposed calculation method is applicable for any drug-protein pair, as long as the corresponding binding constants are known. As an example, phenytoin is known to bind at the indole-benzodiazepine and digitoxin sites of human serum albumin, with binding constants of $1.04 \bullet 10^4$ M^{-1} and $6.5 \bullet 10^3$ M^{-1}, respectively. Normalized phenytoin levels calculated with Equation 1 are displayed in Table 1 (serum albumin and total phenytoin concentrations for patients with altered protein binding were obtained from published data).

Table 1. Calculation of normalized phenytoin levels, based on observed albumin and total phenytoin concentrations

Albumin (μM)	Observed phenytoin (μM)	Normalized phenytoin (μM)
862	43.6	***34.5***
930	41.6	***30.7***
820	48.3	***39.9***
768	45.5	***39.9***
480	72.1	***94.9***
444	32.3	***45.4***
482	35.0	***45.9***
494	37.2	***47.8***
473	34.0	***45.3***
775	85.1	***74.0***

Conclusion: The new model for calculating normalized drug levels is based on binding constants, and is therefore independent on protein or drug concentration. Furthermore, the proposed approach may be applied for any drug-protein binding model. As a direct practical application, the equations can be used for calculating normalized phenytoin and warfarin levels in patients with altered albumin concentration, such as the elderly, trauma patients, and pediatric patients.

47. Kinetics and Isotype-Specificity of Paclitaxel Binding to Microtubules.

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Purpose: An increase in βIII tubulin in cells has been associated with cancer resistance, and studies have shown that taxol has a stronger effect in reducing the dynamics of microtubules in unfractionated microtubules compared to βIII microtubules. The lack of isotype differences within the binding site itself suggests the possibility that isotypes differ instead in residues affecting the ability of paclitaxel to reach its binding site. The purpose of this work is to test this hypothesis and find a means to reverse the isotype specificity. **Methods:** The active site of paclitaxel, which is located within the microtubule's lumen, is believed to be reached by the drug's diffusion through the nanopores in the walls of the microtubule. Moreover it has been suggested that an intermediate step in this process may be the binding of paclitaxel to an intermediate site within the nanopore, from which it moves directly to its active site. We have used computational methods to dock paclitaxel within the nanopore and to simulate its motion to the intermediate binding site, as well as its passage between the two binding sites. **Results:** Using Brownian dynamics simulations, we estimated the rate at which taxol can diffuse to the intermediate binding site found in our docking, and compared this to the experimental rate at which paclitaxel binds microtubules. We generated a targeted molecular dynamics trajectory which seems to show that it is feasible for paclitaxel to use the

H6/H7 loop as a hinge to move directly from the binding site in the pore to the luminal site, as was suggested by Diaz *et al.* (J. Biol. Chem. (2003) 278: 8407). This motion seems to be stabilized by the formation of a hydrogen bond involving a β-tubulin residue which differs in the βIII tubulin isotype as compared to isotypes βI and βII. **Conclusion:** This suggested specific derivatives of paclitaxel which may reverse the isotype specificity, as well as modifications which could lead to an alternate stabilizing hydrogen-bond interaction with tubulin and thus better enable passage to the active site.

48. Characterization of FLJ10324: a novel protein connecting heterotrimeric and small G proteins

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Purpose: In an effort to find novel proteins interacting with the betagamma subunits of heterotrimeric G proteins, we isolated the Gbeta2 protein complex and analyzed its composition using mass spectrometry. Reflecting the success of our approach, several Galpha and Ggamma subunits of heterotrimeric G proteins as well as several subunits of ion channels, which are known interactors of Gbetagamma, were retrieved. One of the most abundant proteins in the Gbeta complex was the uncharacterized protein FLJ10324. The goal of this research is to begin to elucidate the function of FLJ10324. **Methods**: The analyses of the Gbeta2 and FLJ10324 protein complex were performed using liquid chromatography coupled to a mass spectrometry. The mass spectra were then searched against the human NCBI database to identify matching proteins from the peptides. The interaction between Gbeta2, FLJ10324 and Ras family proteins was confirmed using co-immunoprecipitation. Localization of the FLJ10324 protein within cells was performed by generating a fusion protein between the green fluorescent protein and FLJ10324. **Results**: The reciprocal analysis of the FLJ10324 protein complex confirmed the interaction of this protein with Gbeatgamma proteins, several peptides corresponding to different Gbeta and Ggamma subunits were found. Hinting at a possible function for FLJ10324, members of the Ras family of small G proteins including Rap1A, Rap1B, K-Ras, N-Ras were abundant in the FLJ10324 complex. Western blot analyses showed that Rap1a can be co-purified with Gbeta only when FLJ10324 is over-expressed in the cells. Visualization of Venus-FLJ10324 using fluorescent microscopy revealed that FLJ10324 is predominantly a cytoplasmic protein with occasional punctate localization. **Conclusion**: Together these results form the basis of an hypothesis that FLJ10324 may be a novel scaffold protein involved in linking betagamma signalling to small G protein activation.

49. The effect of hyperlipidemia on the biodistribution of halofantrine enantiomers in rat

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Purpose: To examine the influence of hyperlipidemia (HL) on the biodistribution of halofantrine (HF) in the poloxamer 407 model of HL. **Methods:** Normolipidemic (NL) and HL rats (n=4) received 2 mg/kg of HF HCl as bolus i.v. injection through a tail vein. Under isoflurane anesthesia, rats were sacrificed 0.166,1, 3, 6, 12, 24 and 48 h postdose. Specimens were harvested and the concentrations of HF enantiomers in the plasma and tissue homogenates were determined using a validated stereospecific HPLC method**.** The AUC_{0-48} for HF enantiomers were determined in each specimen, and Bailer's method was used to estimate SD from partial AUC to assess significance of differences ($p<0.05$). **Results:** The following mean results were noted for the AUC_{0-48} (μg·h/g):

	Plasma	**Lung**	**Kidney**	**Heart**	**Liver**	**Spleen**	**Brain**	**Fat**
(+)- Halofantrine								
NL	2.37	127	33.6	23.2	56.5	62	0.83	33.7
HL	58.1*	81.5*	32.1	15.4*	66.7*	91.6*	0.71	13.7
(-)- Halofantrine								
NL	1.16	87.1	20.3	13.4	64.8	36.2	0.98	17.0
HL	35.7*	46.5*	17.1	11.7	63.7	53.1*	0.47	6.66
*Denotes significant difference from NL								

HL was associated with significant increases in (+)-HF and (-)-HF enantiomer plasma AUC (25- and 30-fold-respectively). The (+)-HF AUC was significantly increased in HL liver (1.2-fold) and spleen (1.5-fold). In contrast, in HL lung and heart the measured (+)-HF AUC were decreased significantly by 1.6 and 1.5-fold, respectively. Lower (+)-HF mean AUC in HL was noticed in kidney, brain and fat but changes were not significant. For the (-)-HF, there was a significant increase noted in spleen AUC (1.5-fold) and decrease in lung AUC (1.9-fold). All other tissues showed no significant difference in (-)-HF enantiomer distribution. **Conclusions:** The increase in plasma concentration of HF enantiomers in HL did not lead to decreases in all of the tissues. Indeed, the influence of HL on HF tissue distribution was not uniform, but rather enantiomer and tissue-specific. For lipoprotein-bound drugs such as HF, changes in distribution to tissues in response to HL can be complex. **Acknowledgements:** Supported by CIHR grant, MOP 67169.

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50. Development of surface-anchored PLGA nanospheres with mannose-receptor ligands for targeting dendritic cells in cancer immunotherapy

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Purpose: The aim of the present study was to prepare either mannan or O-palmitoyl mannan (OPM) decorated poly (D, L-lactide-*co*-glycolide) (PLGA) nanoparticles and assess the effect of the surface anchorage on the internalization of PLGA nanoparticles by dendritic cells (DCs), that are known to express mannose receptor. **Methods:** Mannan was esterified with O-palmitoyl chloride in the presence of dry pyridine. Prepared OPM was analyzed by ^{1}H NMR, and IR spectroscopy. PLGA nanoparticles were prepared using a water-in-oil-in-water double-emulsion solvent evaporation technique and surface decorated by introducing either palmitoylated mannan or natural mannan in the second water phase. Physical encapsulation of the fluorescent probe, Tetramethylrhodamine Dextran (TMRD), in modified and unmodified PLGA nanoparticles was used to label the nanoparticles. The TMRD loaded nanoparticles were characterized for mean diameter, size distribution and Zeta potential. *In vitro* level of internalization of surface modified nanoparticles by bone-marrow derived DCs was investigated by flow cytometry and fluorescence spectroscopy techniques. **Results:** ^{1}H NMR analysis confirmed the successful synthesis of OPM. The TMRD-loaded nanoparticles anchored with OPM to their surface had mean diameters in the range of 314-383 nm and polydispersity index of 0.11-0.20. The TMRD-loaded nanoparticles modified with natural mannan on the surface showed a slight increase in their mean diameter (379-410 nm) and polydispersity index (0.16-0.22). Assessment of the surface charge showed a zeta potential of -11.9 and -12.4 mv for TMRD-loaded PLGA nanoparticles modified with OPM and blank nanoparticles, respectively. For TMRD-loaded nanoparticles modified with mannan, the zeta potential was -26.0 mv. The flow cytometry studies showed 1.4 and 2.2-fold increase in the level of DC uptake for OPM and mannan decorated PLGA nanoparticles, respectively. This was consistent with the result of fluorescence spectroscopy indicating 1.2 and 2.2-fold increase in the internalization of these nanoparticles. **Conclusions:** Incorporation of mannose-receptor ligands on the surface of PLGA nanoparticles is a promising tool for the development of more efficient cancer vaccines to improve antigen delivery to DCs. **Acknowledgements:** This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC).

51. Isothermal Microcalorimetry and Dissolution Testing to Detect Solid State Reactions in Prednisone Tablets

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Purpose: Solid state reactions between drugs and excipients are detected using either real time or accelerated stability studies. Instabilities or incompatibilities might occur in form of changes in crystalline structure, polymorphism, eutectic mixtures, change in dissolution rate, and lost of water of crystallization. Isothermal microcalorimetry and dissolution testing were used in this study to monitor if two powder mixtures of prednisone with either calcium phosphate dihydrate ($CaPO_4$) and micro crystalline cellulose (MCC) showed any heat flow or differences in dissolution rate after storage at elevated temperature. **Methods**: Prednisone was mixed for 10

minutes using a rotation tumbler (Model 33B, Lortone inc, Mukilteo, WA) with either MCC or $CaPO_4$ in a 1:10 ratio and incubated at 37 °C for four weeks in an isothermal microcalorimeter (TAM III, TA Instruments, New Castle). After incubation, the powder blends were mixed for 10 minutes with either MCC or $CaPO_4$ to obtain similar composed powders. 1% magnesium stearate and 2% croscarmellose sodium were also added. Tablets were compressed at 1 ton compression force. A dissolution test was performed in 500 ml water at 50 rpm immediately after compression using a Varian VK 7020 system equipped with a VK 8000 auto sampler. The samples were analyzed using a HPLC assay. 10 uL media were directly injected onto a Lichrosphere RP60 Select B column. The HPLC system was a Shimadzu 10 A system equipped with a PDA detector. The mobile phase was 65:35 acetonitril : water at 1mL/min. The release profiles were compared using F2 analysis. **Results**: The $CaPO_4$ blend showed a very slight heat flow of -2 μW over four weeks; while the MCC blend did not show any heat flow and was -0.14 μW ±0.8. The comparison of the tablet release profiles showed that both batches had significant different dissolution behaviors with a F2 value of 33.4. After 10 minutes 25.8 % prednisone was released from the incubated $CaPO_4$ powder mix and 16.4 % from the MCC powder mix. After 45 minutes 47.3 and 63.7% prednisone were released respectively. **Conclusion**: Isothermal microcalorimetry as universal heat flow monitoring tool indicates that a solid state reaction between prednisone and $CaPO_4$ occurs. This reaction is presumably responsible for the observed change in dissolution rate. Further studies using DSC and X-ray diffraction are needed to characterize the nature of the change in dissolution behavior of prednisone after incubation with $CaPO_4$.

52. Assessing nanoparticle secondary toxicity mediated by macrophages on non-small lung cancer cells using a two-compartment coculture system

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Purpose: Inhalable nanoparticles (NPs) are of great interest in modern drug delivery; however, data from environmental sciences is concerned about possible toxicity effects. The purpose of this study was to investigate the toxicity of NPs to human non-small lung cancer cells (H460) after being phagocytosed by macrophages, which could be considered as a secondary toxicity. **Methods: A** two-compartment co-culture system was used to evaluate the secondary toxicity of NPs, mediated by macrophages on the H460 cells. A membrane of 0.4 μm pore size was used to separate the two compartments and therefore the two cell lines. H460 cells were grown in the lower compartment. The effect of macrophages after incubation with different concentration of doxorubicin (Dox) solution, blank NP or Dox-loaded NPs on H460 cells was assessed using MTT assay. The effect of concentration, the ratio of macrophages to H460 cells and the co-culturing time was evaluated. To analyze Dox released form macrophages after the phagocytosis of NPs, samples from the lower compartment were collected at different time points and analyzed for free Dox and NP-attached Dox. **Results:** the LD50 of the treatments on macrophages was 25 μg/ml, 250 kcounts/s and 5.0 μg/ml for Dox solution, blank NPs and Dox-loaded NPs, respectively. LD50 was found to be the most suitable concentration to assess the secondary Toxicity. Macrophages to H460 cells ratios between 1:10 to 10:1 were investigated. 10:1 ration showed the highest response rate. Dox solution showed no increase in toxicity between 1, 8 and 24 hours using a concentration range between 5 and 50 μg/ml. Blank NPs and Dox-loaded NPs of an equipotent concentration caused a significant increase in toxicity at 8 and 24 hrs. Dox-loaded NPs showed the highest reduction in H460 cell's viability of 50% after 24 hrs. The analysis of Dox concentration in the lower compartment showed that only 1 μg/ml of Dox was released form macrophages over 24 hrs. This concentration however, is not sufficient to explain the increase in toxicity. **Conclusion:** the two-compartment co-culture system was successfully used to assess the NP's secondary toxicity mediated by macrophages. This toxicity is probably triggered by cytokines release after phagocytosis. Studying changes of cytokines profile should be considered.

53. Synergistic anti-tumor effects of CpG oligodeoxynucleotide and STAT3 inhibitory agent JSI-124 in a mouse melanoma tumor model

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Purpose: The effectiveness of cancer immunotherapy strategies is many cases are hampered by the existence of an intra-tumoral immunosuppressive environment, for which signal transducer and activator of transcription-3 (STAT3) activation in tumor has been shown to be important. Activation of DCs through their toll like receptor (TLR) 9 using unmethylated cytosine phosphate guanine (CpG) oligodeoxynucleotide has been investigated as a strategy for the immunotherapy of cancer. The purpose of this study was to investigate whether CpG-induced activation of DCs in a STAT3-inhibitory environment (induced by JSI-124, a known STAT3 inhibitor) is more effective in suppressing STAT3-active tumors than CpG or JSI-124 alone. **Methods:** B16-F10, a mouse melanoma cell line known to have constitutively active STAT3, was grafted in C57BL/6 mice. Animals were injected intra-tumorally with a) Phosphate buffered saline, b) 10 μg CpG, c) 1 mg/kg JSI-124 or d) 10 μg CpG + 1 mg/kg JSI-124, and monitored for tumor growth and survival. The level P-STAT3 in tumor was assessed after drug administration using immunohistochemistry and Western blot analysis. **Results:** Intra-tumoral injection of JSI-124 alone or in combination with CpG resulted in complete suppression of P-STAT3 level in B16-F10 tumors *in vivo*. Average tumor volume in the mice received JSI-124 + CpG combination therapy was found to be significantly lower than that in the mice treated with either CpG or JSI-124. The combination therapy significantly increased the survival rate of the tumor bearing mice as compared with control and immunotherapy group. C**onclusion:** our findings demonstrate that the combination therapy of a STAT3 inhibitory molecule (e.g., JSI-124) and an immunoadjuvant activator of DCs (e.g., CpG oligodeoxynucleotide) effectively and synergistically suppress tumor growth *in vivo*. **Acknowledgment:** The project was supported by Canadian Institute of Health Research (CIHR). OM was supported by Rx&D HRF Graduate Scholarship in Pharmacy and a scholarship from Iranian Ministry of Health and Medical Education.

54. Therapy of established B16 melanoma tumors by vaccination with melanoma antigen co-encapsulated with adjuvant in PLGA nanoparticles

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Purpose: Previous research in our lab demonstrated the effectiveness of Toll like receptor (TLR) ligand; 7-acyl lipid A as a vaccine adjuvant. We have also reported on the ability of dendrtic cells pulsed with ovalbumin (a model antigen) along with 7-acyl lipid A in poly(lactic-co-glycolic acid) nanoparticles (PLGA-NP) to dramatically enhance ovalbumin specific $CD8^+$ T cell responses. The objective of the present study was to investigate whether a vaccine strategy based on the poorly immunogenic melanoma antigen; Tyrosinase-related protein 2 (TRP2) encapsulated in combination with 7-acyl lipid A in PLGA-NP can elicit *in vivo* antigen specific $CD8^+$ T cell responses that can lead to therapeutic anti-tumor effect. **Method:** B16-F10 cells (10^5 cells/mouse) were implanted subcutaneously (s.c) in the right flank of C57Bl/6 mice. Three days later, animals were randomly assigned to 3 treatment groups (8-10 mice per group). The three groups were s.c injected in the lower flank region with either Empty-NP, TRP2-NP, TRP2-7acyl lipid A-NP. Animals were given booster immunization with the same formulation on day 7 and 13. Mice were monitored for tumor growth; tumor size was measured every 2-3 days. At the end point of the study, animals were euthanized, and individual tumors were isolated and weighted. Detection of IFN-γ secretion pattern of TRP2 specific $CD8^+$ T cells in lymph nodes, spleens and tumors was done using enzyme linked immunospot assay (ELISPOT). Detection of cytokines in the tumor microenvironment was carried out by enzyme linked immunosorbent assay (ELISA). **Results:** Immunization with TRP2-NP slowed down the growth of the tumors compared to what was observed in animals immunized with empty-NPs. There was superior protection effect against tumor growth when 7-acyl lipid A was co-encapsulated with the peptide in the same nanoparticle formulation compared to Empty-NP and TRP2/7-acyl lipid A-NP groups, as evidenced by decreased

tumor size, weight and enhanced antigen specific IFN-γ secretion in lymph nodes, spleens and tumors of the TRP2/7-acyl lipid A vaccinated mice. Particulate delivery of TRP2 and 7-acyl lipid A induced Th1- mediated immune stimulation in the tumor microenvironment as evidenced by increased secretion of TNF-α, INF-γ, IL-2 and IL-12. **Conclusion:** Immunization with PLGA-NP co-encapsulating TRP2 and 7-acyl lipid A induced therapeutic $CD8^+$ T cell-mediated immunity and reversed the immunosuppressive network in the tumor microenvironment.

55. Formulation of inhalable amikacin nanoparticles for the treatment of cystic fibrosis

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Purpose: The purpose of this work was to develop an effective and non-invasive local delivery of amikacin nanoparticles for the treatment of cystic fibrosis. **Methods:** Amikacin nanoparticles were prepared using emulsion polymerization of butylcyanoacrylate monomers. The particle size of the nanoparticles was measured using Zetasizer (Malvern, England). The loading efficiency of nanoparticles for amikacin was performed by centrifuging the nanoparticles in ultracentrifuge for 1 hour and measuring the drug concentration in supernatant fluid. Morphology of the nanoparticles was studied on freeze dried samples using scan electron microscopy technique. The cellular uptake of nanoparticles was evaluated by incubating FITC labeled nanoparticles with a cystic fibrosis tracheal gland cell line, CF-KM4. **Results:** The particle size of nanoparticles was in the range of 115-130 nm. The SEM micrographs showed spherical shape of nanoparticles and confirmed the particle size measured by Zetasizer. The loading efficiency of nanoparticles for amikacin was 75±3.2%. The confocal microscopy pictures showed the accumulation of nanoparticles in the cytoplasm of cells and in some degree in the nucleus of CF-KM4. **Conclusion:** This study showed that the amikacin nanoparticles can be synthesized using cyanoacrylate nanoparticles in proper particles size and high loading efficiency which can accumulate in cystic fibrosis cells. The present study opens a frontier for the development of amikacin nanoparticles which can be delivered locally to the lungs.

56. Lipid-Based Tablet Formulation for Enhanced Oral Delivery of Poorly Soluble Drugs

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Purpose: To develop lipid-based tablet formulations for enhanced oral delivery of poorly soluble drugs. **Method**: Various oils and surfactants were screened for their solubilization effect on the model drug. An optimum lipid combination was then formulated to obtain granules of good characteristics for compression. Based on an increased in-vitro dissolution profile of the tablets with 10% w/w drug load, a tablet formulation with 20% w/w drug load was manufactured and tested in human for exposure. **Results**: Tablets containing Cremophor, propylene glycol and/or Vitamin E TPGS were developed. The absorbents (Cab-o-Sil, ProSolv, and Neusilin) were used to provide suitable compressibility of the lubricated blends. Complete in-vitro dissolution (95% of label claim) was observed for tablets of 10% w/w drug load at 45 minutes in simulated gastric fluid. This was a significant improvement over the only 55% of label claim dissolved with the conventional immediate release tablets of the same strength. Subsequently, tablets with a drug load of 20% w/w were developed in which Cremophor was partially replaced by Vitamin E TPGS for an improved safety profile. In a modified release dose form, this formulation resulted in an increase of ~60% in AUC compared to the non-lipid matrix tablets. **Conclusion:** Compressible granules were obtained from a lipid base, and the prepared tablets showed enhanced exposure compared to a non-lipid based formulation. The study demonstrated the feasibility and advantages of a lipid-based solid dose approach to enhance the oral delivery of poorly soluble compounds.

57. Development and optimization of topical cyclosporin liposomal gel (2% w/w): Double-blind, placebo-controlled clinical trial for efficacy and safety evaluation in patients with stable plaque psoriasis

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Background: Topical delivery of Cyclosporin A (CysA) is highly desirable for various dermatological diseases including psoriasis. However the horny stratum corneum barrier and the unfavorable physicochemical properties of CysA are the major hicupps.(1) Phospholipid-structured liposomes have been successfully used for cutaneous delivery of several therapeutic agents including macromolecules. (2) **Materials & Methods:** Various influential formulation and process variables were screened using Taguchi screening design. D-optimal design was employed to obtain CysA-loaded MLVs with optimum drug-payload (DPL), vesicle size, permeation flux and drug deposition in abdominal mice skin. Optimized CysA liposomes were characterized for their morphological and micromeritic attributes. Rheological and textural properties of the developed liposomal gel were also determined. Clinical efficacy of optimized CysA lipogel was evaluated in 20 patients with symmetrical plaque psoriasis in a 14-week double-blind, placebo-controlled intra-individual comparison study. Safety and tolerability of the developed CysA lipogel was established by monitoring patients' serum CysA levels and pertinent laboratory parameters at periodic intervals. **Results**: Optimized positively charged submicron sized (d50: 0.95 μm) MLVs, composed of unsaturated phospholipid, yielded a DPL of 142.2 μg per mg of total lipids. The results of *in-vitro* skin permeation studies demonstrated that these cholesterol-free liquid state MLV's could effectively localize CysA molecules in the skin layers. Developed lipogel showed thixotropic behavior with excellent spreadibility. Results of clinical study employing formulated CysA lipogel (2.0% w/w) indicated marked improvement in clearance of psoriatic lesions vis-à-vis placebo lipogel. Total clearance of lesions was observed in 12/20 patients with once daily application of CysA lipogel for 14 weeks. In all patients, the serum CysA levels were quite low (i.e., below the detection limit) throughout the 14 weeks of continuous use. No clinically significant changes in blood or other laboratory parameters were seen. Finding the CysA lipogel formulation to be cosmetically acceptable, all the patients were willing to use it in future as well. **Conclusions:** Promising outcomes of our investigations unequivocally vouch for the plausibility of this effective and safe topical lipogel of CysA as an important drug delivery tool for the management of dermatological disorders *viz.* psoriasis. **References:** 1) Madan, V. and Griffiths, C. E. 2007. Systemic ciclosporin and tacrolimus in dermatology. Dermatol Ther. 20: 4: 239-50. 2) Barry, B. W. 2004. Breaching the skin's barrier to drugs. Nat. Biotech. 22: 165-167.

58. Preparation of thermoresponsive hydrogels for regional drug delivery

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Purpose: The objective of this study is to design biodegradable and thermoresponsive hydrogels based on triblock copolymers of poly (ethylene glycol) (PEG) and poly (ε-caprolactone) (PCL) and assess the potential application of developed hydrogels as injectable implants or topical drug delivery systems. **Methods:** Two types of triblock copolymers, i.e., PEG-*b*-PCL-OCO $(CH_2)_4$OCO-PCL-*b*-PEG and PCL-*b*-PEG-*b*-PCL, were synthesized using different PEG and PCL molecular weights. For the synthesis of the first class of triblock copolymers, monohydroxylated PEG-*b*-PCL diblock was coupled to another monohydroxylated PEG-*b*-PCL diblock, using adipoyl chloride as coupling agent. PCL-*b*-PEG-*b*-PCL was synthesized by ring-opening polymerization of ε-caprolactone using dihydroxylated PEG as the initiator. The molecular weight and polydispersity of the resultant copolymers were determined by ^{1}H NMR, and GPC. The phase transition at different polymer concentrations and temperatures was followed using test tube inversion method and differential scanning calorimetry (DSC). The rheological behavior of prepared gels at room temperature and 37◦C were studied by Dynamic Stress Rheometer. The effect of hydrophobic/hydrophilic molecular weight ratio of triblock copolymers on transition behavior was assessed. The release profile of celecoxibe from selected hydrogel to a recipient phase of 30% (w/v) sodium dodecyl sulfate (SDS) in water was determined by FRANZ diffusion cells using cellulose filter paper at 37^0C.

Results: The ^{1}H NMR and GPC results confirmed the successful preparation of triblock copolymers. Tri block copolymers of PCL-*b*-PEG-*b*-PCL displayed gel to sol transition behavior as the temperature was raised. An increase in the hydrophobic/hydrophilic content of the block copolymer raised the gel to sol transition temperature of block copolymers in the range of 20-50^{0}C as confirmed by both tube inversion and DSC methods. Celexocibe displayed a sustained released behavior up to 12 hours at 37^{0}C when loaded in PCL-*b*-PEG-*b*-PCL hydrogels. PEG-*b*-PCL-OCO $(CH_2)_4$OCO-PCL-*b*-PEG block copolymers displayed sol to gel transition behavior as a result of an increase in temperature. Among different polymers under study, the closest sol to gel transition temperature to body temperature was observed for PEG-*b*-PCL-OCO $(CH_2)_4$OCO-PCL-*b*-PEG triblock copolymers with a PEG/PCL ratio of 0.6 (750 Da PEG and 1200 Da PCL) at 45^{0}C. **Conclusion:** The results points to a potential of biodegradable hydrogels made from tri block copolymers of PCL-*b*-PEG-*b*-PCL and PEG-*b*-PCL-OCO$(CH_2)_4$OCO-PCL-*b*-PEG as topical drug delivery systems and injectable implants for sustained regional drug release, respectively.

59. Bioequivalence assessment of topical clobetasol propionate products using visual and chromametric assessment of skin blanching

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Purpose: The assessment of the degree of skin blanching following the application of a formulation containing a topical corticosteroid has been established as a surrogate method for the determination of bioequivalence of such topical preparations. Whereas initially the procedure involved visual assessment of the blanching response, an instrumental procedure using a chromameter was subsequently recommended as the method of choice in such evaluations. In this study, both visual and chromametric assessments have been carried out on two topical creams containing clobetasol propionate (0.05%) and the results from both methods are compared and discussed. **Methods:** Human subjects (volunteers) were subjected to screening using a cream containing 0.05% clobetasol propionate, in order to identify appropriate subjects for inclusion in the study. Subsequently the study was implemented according to the FDA guidance using both visual and chromameter assessment techniques. Blanching responses were assessed visually by three trained, independent observers and instrumentally using a Chromameter®. An ED_{50} of 36 min was used as the dose duration based upon data previously obtained from a pilot study using the same topical corticosteroid reference product. A visual rating scale of 0–4 and the a-scale readings from the chromameter were used. **Results:** The visual and chromameter blanching profiles showed similar blanching responses and corresponded well with each other. The 90% confidence interval for the visual and chromameter data were calculated using Locke's method and when only the data obtained from 23 subjects who were identified as "detectors" (according to the FDA guidance) were used, the products fell within the bioequivalence acceptance range of 80-125% using the visual assessment method (99.3-111.6%) whereas the data using a chromameter (86.5-129.3%) were just outside the acceptance limits. However, when all subjects (n=34) were included in the calculations, both the visual (97.9-109.2) and chromameter (90.2-120.7) data fell within the acceptance range for the declaration of bioequivalence. **Conclusions:** Whereas visual data indicated bioequivalence using either data from "detectors" or data from all subjects, the chromameter data from "detectors" only indicated bioinequivalence but inclusion of all subject data fell within the acceptance range to be declared bioequivalent. The data indicate the utility of visual assessment of skin blanching for the assessment of the bioequivalence of topical corticosteroid products. **Acknowledgements:** Funding from the South African National Research Foundation (NRF) and the Biopharmaceutics Research Institute (BRI) is gratefully acknowledged.

60. Harnessing Mitochondria-Penetrating Peptides for Delivery of Therapeutic Nucleic Acids

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Purpose: Mitochondria, essential in metabolism and apoptosis, are implicated in a number of pathologies, including diabetes and cancer. These organelles possess their own genome and mutations in these sequences are associated with specific mitochondrial disorders. Currently, the lack of targeting methods to transport drugs or therapeutic agents into this organelle greatly impedes the study and treatment of mitochondrial disease. **Methods***:* Our lab has recently designed a tunable peptide scaffold and through systematic chemical modifications, we have elucidated the precise chemical properties required for mitochondrial localization. To evaluate uptake across the plasma membrane and organellar localization of these peptides, we utilized flow cytometry and confocal microscopy, respectively. **Results***:* Through these studies, we have successfully identified the precise lipophilicity and cationic thresholds required for mitochondrial localization and efficient uptake. These mitochondria-penetrating peptides (MPPs) are now being harnessed to deliver nucleic acids to the mitochondria. Using our knowledge on the chemical properties required for localization to this organelle, we have successfully utilized these peptides to deliver nucleic acid mimics to the mitochondria. DNA analogs, including peptide nucleic acids (PNAs), are being used to inhibit protein expression from mutated mitochondrial genome sequences. This PNA system will be adapted to bind mutant DNA and tRNA sequences associated with different disease states. Through the selective inhibition of expression from these mutant sequences, phenotypic rescue will be achieved. **Conclusion***:* Harnessing MPPs and our knowledge of chemical properties required for mitochondrial localization will allow us to deliver functional therapeutics to this organelle for the further study and treatment of mitochondrial disorders.

61. Effects of intravenous and subcutaneous administration on the pharmacokinetics, biodistribution, cellular uptake and immunostimulatory activity of CpG ODN encapsulated in liposomal nanoparticles.

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Purpose: We have previously demonstrated that the immune response to an unmethylated cytidine-guanosine (CpG)-containing oligonucleotide (ODN) is greatly enhanced when encapsulated in a lipid nanoparticle (LN-CpG ODN). In particular, encapsulation takes advantage of the natural ability of antigen presenting cells (APCs) to accumulate LN-CpG ODN, resulting in increased immune cell activation. In this study, the pharmacokinetics, biodistribution and cellular uptake of LN-CpG ODN following intravenous (i.v.) and subcutaneous (s.c.) administration is characterized and correlated with immunostimulatory activity. **Methods:** Pharmacokinetic and biodistribution studies were performed in ICR mice injected i.v. or s.c. with free or encapsulated ^{3}H-ODN at a dose of 20mg/kg or 5mg/kg, respectively. Uptake studies were performed in ICR mice injected i.v. or s.c. with 10 mg/kg FITC-labeled ODN encapsulated within lipid particles and cell suspensions were analyzed for uptake by flow cytometry. Activation and cytotoxicity assays were performed in C3H mice injected i.v. or s.c. with free or encapsulated ODN at a dose of 20mg/kg. For activation studies, splenocytes and PBMCs were stained with fluorescently labeled antibodies against phenotype and activation markers, and analyzed using flow cytometry. For cytotoxicity studies, NK-specific killing was assessed in a standard 4 hour ^{51}Chromium release assay using YAC-1 cells as targets. Activation of antibody-dependent cell-mediated cytotoxicity was assessed in a similar manner using Daudi cells in the presence/absence of the monoclonal antibody Rituxan (100ug/10^6 cells at a concentration of 10ug/ml). **Results:** It is shown that, despite dramatic differences in tissue distribution profiles and considerable differences in uptake by APCs following i.v and s.c. administration, the resultant immune response is very similar with respect to levels of cellular activation and cytolytic activity (NK cells and monocytes/macrophages) in the spleen and blood compartments. **Conclusion:** In conclusion, results presented here indicate that LN-CpG ODN elicits similar responses following i.v. and s.c. administration in spite of dramatic differences in biodistribution. It is concluded that the inherent ability of APCs to accumulate liposomal nanoparticles results in very efficient uptake of LN-CpG ODN, even when present at very low concentrations, resulting in potent immune cell activation. Our

findings demonstrate that in comparison to free CpG ODN, LN-CpG ODN offers the dual benefits of significantly enhanced immune responses as well as a relative insensitivity of the immune response to route of administration. **This work has previously been published in:** International Immunopharmacology 7 (2007) 1064-1075.

62. Phagocytosis of PLGA microspheres containing rifampicin by alveolar macrophages for overcoming tuberculosis

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Purpose: Tuberculosis is one of the most serious intractable infectious diseases worldwide. To overcome tuberculosis, the delivery of microspheres (MS) loaded with antituberculosis agents to alveolar macrophage (AMϕ) infected with *Mycobacterium tuberculosis* (MTB) is expected to be effective, because MTB invading the lungs during respiration is liable to be captured by AMϕ cells. We prepared the microspheres of poly(lactic-co-glycolic) acid (PLGA) containing the antituberculosis agent rifampicin (RFP-PLGA), and examined their phagocytic uptake by AMϕ cells. **Methods:** RFP-PLGA MS were prepared by dissolving PLGA and RFP in dichloromethane used as an oil phase. Then, the solution was injected into 2.0%(w/v) polyvinyl alcohol (PVA) aqueous solution through Shirasu porous glass membranes. The cell line NR8383, which is derived from rat AMϕ, were used as a model of AMϕ. NR8383 cells were cultured in F-12K medium supplemented with 15% heat-inactivated fetal bovine serum (FBS) and 60 μg/mL ampicillin at 37 °C under the stream of 5% CO_2. Then, cells (5 x 10^5 cells/mL/well) were incubated with RFP-PLGA MS at 37 °C for various periods. After incubation, the population of phagocytic AMϕ cells, and the number of MS taken up by AMϕ cells were counted in optical microscopic fields. **Results:** RFP-PLGA MS were well phagocytosed by AMϕ cells resulting in high accumulation of RFP in AMϕ cells. The amount of RFP inside AMϕ taken up by phagocytosis of RFP-PLGA MS was about 20 times greater than that administered in a form of solution. The phagocytosis of RFP-PLGA MS was dependent on the size of MS. It was found that the 3-μm RFP-PLGA MS were optimal to attain the high population of phagocytic AMϕ cells and high number of MS phagocytosed by individual AMϕ cells. Interestingly, phagocytic activity of AMϕ cells was enhanced by phagocytosis of RFP-PLGA MS. Phagocytosis of RFP-PLGA MS was not toxic to AMϕ cells, and killed effectively the *Mycobacterium bovis* Calmette-Guérin (BCG), used as a model of MTB, residing in the AMϕ cells. **Conclusion:** The present results shown above suggest that the delivery of RFP-PLGA MS into AMϕ cells infected with MTB is promising for clinical treatment of tuberculosis. The study on the pulmonary delivery of RFP-PLGA MS by insufflation in rats and mice are under way.

Natural Products

63. Peganum harmala L.; a candidate herbal plant for treatment of dioxin mediated carcinogenicity

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Purpose: Dioxins are widespread environmental contaminants that have been linked with a variety of deleterious effects on human health including increased cancer rates. The detrimental effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, one of the most common environmental dioxins) are mediated via the aryl hydrocarbon receptor (AhR), a transcription factor that regulates the expression of the carcinogen-activating enzymes cytochromes P450 1a1 (Cyp1a1). In the present study, we examined the ability of *Peganum harmala* L. (Zygophyllaceae), a widely used medicinal plant in Middle East and North Africa, to affect TCDD-activated AhR-mediated signal transduction in mouse hepatoma Hepa 1c1c7 cells. **Methods:** P. harmala fruiting tops were dried in shade, ground, extracted using methanol, concentrated under reduced pressure and finally freeze dried to remove any traces of solvent and to improve solubility. The cytotoxicity of plant extract was assessed using the MTT assay. The effect of different extract concentrations on Cyp1a1 mRNA and protein levels were determined using real time-polymerase chain reaction and Western blot analysis, respectively. Cyp1a1 catalytic activity was determined using 7-ethoxyresorufin as a substrate. To determine whether the effect of plant extract is an AhR ligand dependent, we examined the effect

of plant extract on Cyp1a1-induction-mediated by three different AhR ligands namely; benzo(a)pyrene, 3-methylcholanthrene, and β- naphthoflavone. The role of AhR-dependent mechanism was determined using Hepa 1c1c7 cells transiently transfected with the XRE-driven luciferase reporter gene**. Results:** Our results showed that P. harmala methanolic extract had no apparent cellular toxicity effects up to 50µg/ml. In addition, the plant extract significantly inhibited the TCDD-mediated induction of Cyp1a1 at mRNA, protein and activity levels. Similar pattern of inhibition at the catalytic activity level was observed with the other AhR ligands tested, suggesting that the inhibitory effect of P. harmala methanolic extract is AhR ligands independent. The ability of P. harmala methanolic extract to inhibit Cyp1a1 was strongly correlated with its ability to inhibit AhR-dependent luciferase activity, suggesting that AhR-dependent mechanism is involved. **Conclusion:** This is the first demonstration that Peganum harmala L. can prevent AhR ligands-mediated carcinogenicity. These results raise the possibility that P. harmala may be a promising candidate for treatment of dioxin and other AhR ligands-induced carcinogenicity. **Acknowledgements:** This work was supported by the Natural Sciences and Engineering Council of Canada (NSERC) Grant RGPIN 250139 to A.O.S.E. M.E. is the recipient of Egyptian Government Scholarship.

64. Photoprotective and photochemical properties of quercetin

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A variety of plants such as *Brassica* and *Arabidopsis* are known to respond to high levels of UVB radiation with an increased biosynthesis of polyphenols such as quercetin, whose role is believed to be that of a photoprotectant. Our interest is in the use of topically applied quercetin to prevent the damaging effects of UV radiation to skin such as carcinomas and photoaging. **Purpose:** The purposes of this investigation are to determine: 1) the stability of quercetin to UVR and identify degradation products, 2) the ability of quercetin to inhibit UV-induced oxidative stress, 3) the ability of quercetin to inhibit UV-induced markers of skin damage in a model skin system. **Methods:** The ability of quercetin to prevent lipid peroxidation has been assessed using an *in vitro* model of lipid peroxidation. Quercetin's ability to prevent the induction of the UVA response protein MMP-1 is being assessed using ELISA. Development of an HPLC-DAD/MS-MS technique for the determination of thymine dimers (a UVB endpoint) and 8-oxo-guanosine (a UVA endpoint) is currently underway. Photostability and photodecomposition products of qercetin have been determined using spectroscopic techniques. **Results:** Quercetin was determined to have an antioxidant stoichiometric ratio of 4, indicating a high antioxidant potential. This value was identical for both AAPH- and UVB-induced lipid peroxidation, suggesting that quercetin inhibits lipid peroxidation through a radical-scavenging pathway rather than absorption of UVB radiation. The stoichiometric ratio of quercetin against UVA induced lipid peroxidation was found to be 2, suggesting that quercetin may act as a pro-oxidant in the presence of UVA radiation. Preliminary studies have shown that 100µM quercetin can reduce the levels of MMP-1 found in the extracellular space of UVA and UVB exposed human keratinocytes. Quercetin has been found to be stable under exposure to both UVA and UVB radiation, with only three photoproducts appearing at low yields after a minimum of 2 hours exposure unless a triplet state sensitizer in included. Structural analysis of the photoproducts suggests decomposition through cleavage of the C-ring. **Conclusion:** Preliminary results indicate that quercetin is stable under UV light and is effective in preventing lipid peroxidation and release of MMP-1 in keratinocytes. Studies to determine the effectiveness of quercetin in preventing pre-cancerous thymine dimer and 8-oxo-guanosine DNA lesions in keratinocytes are currently underway.

65. Evaluation of Cree botanicals for their role to potentiate cytotoxicity in human C2BBe1 intestinal cells

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Purpose: To evaluate six Cree botanicals, used by the Cree of Eeyou Istchee to treat type II diabetes (T2D), for their role to potentiate cytotoxicity in human C2BBe1 intestinal cells and evaluate one particular botanical, AD09, for its role in modulating gene expression as measured by microarrays. **Methods:** Human C2BBe1 intestinal cells, clone of Caco-2 cells, were exposed to concentrations ranging from 10 □g/mL to 200 □g/mL of ethanolic extracts obtained from six botanicals (AD01, AD07, AD08, AD09, AD11 and W2) for a period of 24 hours. Cytotoxicity

levels were then measured through Promega's CytoTox 96® Non-Radioactive Cytotoxicity Assay, which examines the release of lactate dehydrogenase. Furthermore, C2BBe1 cells were exposed to AD09 at a concentration of 100 □g/mL, 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. **Results:** Out of the six botanicals examined for cytotoxicity, only AD11 showed moderate to significant toxicity at concentrations of 150 □g/mL and 200 □g/mL. The other extracts appear to exhibit minimal to no toxicity in the C2BBe1 intestinal cells. Microarray results for AD09 will be presented. **Conclusions:** Extract concentrations of 150 □g/mL and 200 □g/mL are extremely high and such levels would not be achieved in the body, thus we can speculate that these extracts will not pose an immediate toxicity danger, especially when it comes to first-pass metabolism, for which the C2BBe1 cells are commonly used to study. However, any possible gene transcript changes, particularly to the cytochrome P450 metabolic enzymes, that may occur can lead to possible flags for drug interaction, since many drugs are metabolized by these enzymes. T2D patients usually take many concurrent medicines along with the plants, thus there is potential for adverse drug interactions and so further evaluation of these botanicals is needed.

66. Effect of Cree anti-diabetes plants extract on Phase I drug metabolism enzymes

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Purpose: The Cree Nation of Eeyou Istchee (CEI) of Northern Quebec has a high prevalence of Type II diabetes. A number of herbal medicines have been used for treating diabetes symptoms in the CEI and several have promising antidiabetic activity in in vitro bioassays. However, their metabolism profiles and capability to interact with conventional therapeutic product are not known. Some Cree anti-diabetic plant products may affect drug metabolism, potentially affecting the efficacy and safety of drugs or other herbal medicines. The purpose of this study is to examine the effect of these Cree products on cytochrome P450 (CYP) 3A4, 19 and human flavin-containing monooxygenase (FMO) 3-mediated metabolism. **Methods:** Ethanol extracts were made from 19 source materials: AD01, AD02, AD03, AD06, AD07, AD08, AD09, AD11, AD12, AD13, W1, W2, W3, W4, W5, W6, W7, W8, and W9, then dissolved in methanol (10 mg/ml) and examined for their effect on CYP19, 3A4, 4A11 and FMO3 activity by using *in vitro* bioassay. **Results:** Our data indicates that methanolic extracts of all 19 Cree products show some degree of inhibition ranging from low (0-35%) to high (70-99%). Of note, the methanolic extracts of AD02, AD11, AD12 and D13 produced marked inhibitory effects on both CYP3A4 and FMO3, while W6, W7 and W9 showed low inhibition effect on both enzymes. **Conclusions:** Some of the Cree products examined have pharmacological properties that have the potential capacity to affect the safety and efficacy of drugs and other health products, and may also have the potential capacity to interfere the metabolism of some endogenous substrates. Further studies are warranted against a wider range of Phase I drug metabolism enzymes and to determine if these effects are clinically significant.

Clinical Sciences

67. An Interim Analysis: Nasal Colonization of *Staphylococcus aureus* in Relapsing-Remitting Multiple Sclerosis Patients

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Background: Epidemiologic studies have led some investigators to suggest that environmental factors, such as infectious diseases, may be associated with the underlying pathogenesis of multiple sclerosis (MS). This theory has arisen from the association between geographical location and 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 *Staphylococcus aureus* (*S. aureus*). 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 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 significant correlation 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 naive 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 = 41) 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 the province in which they reside.

Pharmaceutical Chemistry

68. Novel autoxidation product of nordihydroguaiaretic acid

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Purpose: Nordihydroguaiaretic acid (NDGA) is a naturally-occurring lignan which has been used in traditional medicines and has been studied for a number of pharmacological properties such as induction of apoptosis, growth inhibition of β-amyloid fibrils and its anti-cancer properties including cancer chemoprevention. Chronic use of the NDGA-containing herbal "chaparral" however, is known to be hepatotoxic. NDGA autoxidizes rapidly at alkaline pH to reactive electrophiles which can be trapped by glutathione. The purpose of this study was to identify the products from the autoxidation of NDGA in the absence of glutathione and determine what their contribution is to the NDGA-mediated inhibition of cytochrome P450s *in vitro*. **Methods**: NDGA was allowed to oxidize at pH 8.0 and the major product was purified by normal-phase and reverse-phase flash column chromatography. Structural elucidation was carried out using MS, UV and NMR (^{1}H, ^{13}C, COSY and NOESY) experiments. Cytochrome P450 inhibition studies were carried out in pooled rat liver microsomes using methoxyresorufin (resorufin: 1A2) and testosterone as probe substrates (testosterone 6β-OH: 3A4, 16α-OH: 2B/2C11 and 2α-OH: 2C11). **Results**: A novel schisandrin-like cyclolignan was determined to be the major product of NDGA autoxidation in the absence of glutathione. A mechanism involving radical coupling of a carbon-centred, resonance-stabilized di-radical is consistent with the observed product. Inhibition studies of cytochrome P450 1A2, 2B, 2C11 and 3A indicate that the NDGA cyclolignan is an inhibitor of 1A2, 2C11 and 3A, compared with NDGA which was an inhibitor of 1A2, 2B/2C11 and 3A. **Conclusions**: An NDGA cyclolignan is formed rapidly at neutral pH and may be responsible for some of the biological activity associated with NDGA. Bioassay conditions, including incubation time and pH, will be important for determining the actual active species present in other biological assays.

69. Rational Design and Cytotoxicity of Novel Colchicine Derivatives

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Purpose: Targeted molecular medicine is an exciting research approach to develop safer and more effective new chemotherapeutic agents. Because the β-tubulin protein is already a successful anticancer drug target for a number of anti-mitotic compounds, using computationally determined physical characteristics of tubulin to develop novel and effective drugs that have minimal side effects is an exciting prospect The existence of several isotypes of β-tubulin, coupled with their varied distribution in both normal and cancerous cells has provided us with a platform upon which to construct novel chemotherapeutic agents that are able to differentiate between these cells. A drug that specifically targets those tubulin isotypes expressed in cancer cells would maintain its cytotoxic activity on these cells, yet have a reduced effect on cells found in normal tissues. **Methods**: To test this hypothesis, we have performed homology modeling on a consensus set of nine human β-tubulin isotypes and analyzed them for differences in the colchicine-binding site. Using this binding cavity as a template, we have designed several novel colchicine derivatives, which we computationally probed for binding affinity to β-tubulin using molecular modeling experiments. Colchicine derivatives were prepared by the acylation of the common intermediate 1-demethylcolchicine, which afforded esther derivatives while alkylation gave ether derivatives. Cell lines used in this study included CEM, MCF-7, HeLa, and A549, MOO6X, MO10B (glioma cell lines) and Jurkat for cytotoxicity analysis. Cytotoxicity testing was completed using a common cell viability assay, the MTS assay. **Results**: Once a set of diverse colchicines derivatives had been identified, a small subset was synthesized and tested against a number of cancerous cell lines. The colchicine derivatives could be divided into two groups, depending on the type of substitution, ether or ester, on the A-ring. After the assay of the first set of compounds we focused only on more promising ether derivatives. Cytotoxicity results for all of the colchicine derivatives correlated nicely with calculated binding affinities **Conclusion**: Cytotoxicity assays were performed, yielding a correlation to computational binding predictions. These results demonstrate the utility of computational screening in the design of colchicine that may be able to differentiate tubulin isotypes. Tubulin isotype specific drugs should have fewer side effects, as they will bind to and disrupt microtubules only in cells expressing a particular isotype.

70. Synthesis of analogs of NDGA and their oxidative metabolism

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Purpose: The purpose of this study is to investigate the oxidative metabolism of analogues of nordihydroguaiaretic acid (NDGA) (Figure 1). NDGA is a naturally-occurring di-catechol lignan which is under study for both its pharmacological and toxicological properties. Catechols may be oxidized to either *ortho*-quinones or *para*-quinone methides. NDGA rapidly autoxidizes under alkaline conditions to produce an *ortho*-quinone but the *para*-quinone methide is not formed. The toxicity of NDGA is believed to be a function of its oxidative metabolism to an *ortho*-quinone species. We are preparing these analogues in an effort to determine why NDGA autoxidation occurs so rapidly and what structural features prevent *para*-quinone methide formation in NDGA. **Methods**: Analogues of NDGA are being prepared for oxidative metabolism studies. A synthetic strategy that allows preparation of a variety of analogues was adopted. Our strategy employs consecutive Stobbe condensations to form the lignan skeleton. A monocatechol analogue of NDGA (Figure 2) has been prepared as a model compound for oxidative metabolism studies using this approach. Oxidative stability of the analogue under alkaline conditions will be determined by monitoring the loss of starting material by HPLC. The analogue will be oxidized in rat liver microsomes in the presence and absence of glutathione and analyzed by HPLC-UV. Resulting products will be compared to standards of glutathione adducts prepared by oxidizing the lignan with tyrosinase, and with silver oxide. All compounds will be identified using standard spectroscopic techniques. **Results**: The monocatechol analogue of NDGA was successfully prepared via two Stobbe condensation reactions between diethyl succinate and the appropriate units of benzaldehyde. The resultant alkene was hydrogenated to the alkane and the ester groups

converted to methyls via reductive methods giving the desired final product, with individual yields in the nine-step process ranging from 60%-98%. **Conclusions**: Our synthetic method is an effective technique for the synthesis of structural analogues of NDGA, and allows for the facile introduction of different functional groups on the lignan skeleton.

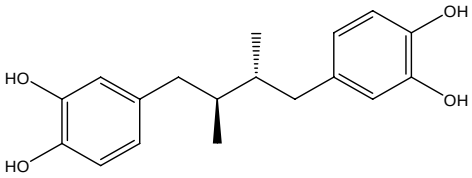

Figure 1: NDGA

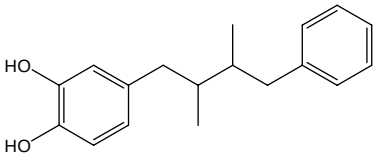

Figure 2: Monocatechol Analogue of NDGA

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