Canadian Society for Pharmaceutical Sciences
Societe canadienne des sciences pharmaceutiques

CSPS 6th Annual Symposium
on Pharmaceutical Sciences

May 28-31, 2003
Delta Centre-Ville
Montréal, Québec

The Science of Drug Discovery & Development

CSPS

Journal of Pharmacy & Pharmaceutical Sciences

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CAPRION

6th Symposium programme - The science of drug dscovery & development

Wine & Cheese Welcoming Reception, Wednesday, May 28, 1800h-1900h, Foyer, Regence B, Floor C, (sponsored by **Anapharm**)

Exhibitors, Wednesday, Thursday, Friday, May 28, 29, 30, 0900h - 1600h, Foyer, Regence BC, Floor C

CSPS Programme

Thursday, May 29

0800h-1600h *CSPS Poster Presentations*, Foyer, Regence BC

0830h-1610h *Symposium*, Regence A, Floor C

0830h *Welcome*, Programme Chair: Elizabeth Vadas, INSCITECH Inc., Dorval, Québec, Canada.

0835 *Merck Company Foundation Poster Presentations* by Undergraduate Summer Studentship Programme Research Award Recipients. Kishor Wasan, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada

Session 1: *Impact Of Rational Drug Design On Pharmaceutical Development.* Chairs: Lakshmi Kotra, University of Toronto, The Leslie Dan Faculty of Pharmacy and Department of Chemistry Molecular Design and Information Technology (MDIT) Center, Ontario, Canada; Gordon McKay, PharmaLytics Inc., Saskatoon, Saskatchewan, Canada.

0845h *Impact Of Targeted Libraries In Drug Discovery*. Michael Bös, Boehringer Ingelheim (Canada) Ltd., Research & Development, Laval, Quebec, Canada

0920h *Impact Of NMR/Structural Biology In Drug Discovery/Development*. Stephen Fesik, Abbott Laboratorie, Chicago, Illinois, USA

0955h **Coffee/Tea Break.** Meet the CSPS poster presenters.

1025h *The Critical Role Of Informatics In Exploratory Drug Development*. Mark Powell, Bristol-Myers Squibb Company, ARD, Princeton, New Jersey, USA

1100h *Physicochemical Properties In Drug Design/ Development*. Christopher A. Lipinski, Pfizer Global Research & Development, Groton Labs, Connecticut , USA

1135h Panel Discussion

1200h **Box Lunch Buffet,** Foyer, Regence ABC. Meet the CSPS poster presenters.

Session 2: *Pharmacokinetics, Preclinical ADME, Toxicology.* Chairs: Elizabeth Kwong, Pharmaceutical Research and Development, Merck Frosst Canada & Co., Kirkland, Québec; Kishor Wasan, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada.

1300h *Modeling Of Intestinal Drug Absorption And Bioavailability*. K. Sandy Pang, Faculty of Pharmacy, University of Toronto, Ontario, Canada

1340h *The Pharmacokinetics Of Drug Transfer In Humans And Chronically Instrumented Sheep*. Wayne Riggs, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada

1420h **Coffee/Tea Break.** Meet the CSPS poster presenters.

1450h *Non-clinical Toxicology/Safety Studies To Support Initiation & Continuation Of Clinical Trials* .Glenn Washer, Toxicology & Safety Assessment, Theratechnologies, Inc., Montréal, Québec, Canada

1530h *Prediction Of Plasma And Tissue Pharmacokinetics Of Drugs In Animals And Human Using In Silico And In Vitro Data As Sole Input In Generic Physiologically-Based Pharmacokinetic (PBPK) Models*. Patrick Poulin, Biomodeling & Simulation group, F. Hoffmann-La Roche Ltd., Basel, Switzerland

1610h *CSPS Members Annual General Meeting, Cartier AB, Floor C*

Friday, May 30

0800h-1600h *AFPC Poster Presentations*, Foyer, Regence BC

0830h-1550h *Symposium*, Cartier AB, Floor C

Session 3: *Biomarkers As Surrogate Endpoints In Preclinical And Clinical Development.* Chairs: Pollen K.F. Yeung, College of Pharmacy, Dalhousie University, Halifax, Nova Scotia, Canada; William Casley, Centre for Biologics Research, Health Canada, Ottawa, Ontario, Canada.

0830h *Opening Remarks*. Chairs: Pollen K.F. Yeung, William Casley

0845h *Current Drug Discovery Programmes At Merck Frosst: Bridging Drug Discovery To The Clinic With Surrogate Markers*. Kathleen Metters, Centre for Therapeutic Research, Merck Frosst Canada & Co., Kirkland, Quebec, Canada

0920h *Integrating Preclinical And Clinical Evidence: Linking Drug Pharmacology And Empiricism*. Terrence Blaschke, Department of Medicine, Division of Clinical Pharmacology, Stanford University School of Medicine, California, USA

0955h **Coffee/Tea Break.** Meet the AFPC poster presenters.

1025h *Comprehensive difference mapping and protein identification in biomarker studies*. Daniel Chelsky, Caprion Pharmaceuticals Inc., Montréal, Québec, Canada

1100h *Regulatory Perspective On Biomarkers As Surrogate Endpoints In Preclinical And Clinical Development*. Agnes Klein, Health Canada, Clinical Evaluation Division, Biologics and Genetic Therapies Directorate, Ottawa, Ontario, Canada

1135h Panel Discussion

1200h **Box Lunch Buffet**, Foyer, Regence ABC. Meet the AFPC poster presenters.

Session 4: *Intersubject Variability In Drug Response*. Chairs: Fakhreddin (Mo) Jamali, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada; Jacques Turgeon, Faculty of Pharmacy, University of Montreal, Montréal, Québec, Canada

1300h *Intersubject Variability In Drug Action*. Jacques Turgeon, Faculty of Pharmacy, University of Montreal, Montréal, Québec, Canada

1335h *Pathophysiological Changes And Intersubject Variability*. Fakhreddin (Mo) Jamali, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

1410h *Natural Health Products And Drug Disposition*. Brian Foster, Therapeutic Products Directorate, Health Canada, Ottawa, Canada

1445h **Coffee/Tea Break**. Meet the AFPC poster presenters.

1515h *Multiple Mechanisms By Which Grapefruit Juice Alters Drug Disposition And Response In Humans*. David Bailey, Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada

1800h *CSPS Dinner & Awards Banquet*, Regence B, Delta

Saturday, May 31

0830h-1200h *Symposium*, Auditorium, Mezzanine

Session 5: *Novel Excipients And Formulation Technologies.* Chair: Jasmine Prucha, Gattefossé Canada Inc., Toronto, Ontario, Canada; Amyn Sayani, GlaxoSmithKline, Inc., Mississauga, Ontario, Canada; Programme Chair: Elizabeth Vadas, INSCITECH Inc., Dorval, Québec, Canada

0830h *Opening Remarks*. Chairs: Jasmine Prucha, Amyn Sayani

0840h *Lipid-based Vehicles For Oral Delivery*. Duncan Q.M.Craig, Biophysical Pharmacy, School of Pharmacy, Queen's University, Belfast, Ireland

0920h *Formulation Development Of New Chemical Entities Using Special Excipients And Technologies*. Kwok Chow, Formulation Development, Pharmaceutical Development Services, Patheon, Inc., Mississauga, Ontario, Canada

1000h **Coffee/Tea Break**.

1030h *New Developments For The Application Of Nanoparticle Technology To Drug Discovery And Development*. Eugene Cooper, Research and Development, Elan Drug Delivery Inc., King of Prussia, Pennsylvania, USA

1110h *Overview Of Novel Oral Drug Delivery Systems: Rapid Dissolve And Controlled Release*. Sophie-Dorothée Clas, Pharmaceutical Research & Development, Merck Frosst Canada & Co., Kirkland, Quebec, Canada

1150h *Use Of Permeation Enhancer Excipients In Oral Formulations: A Case Study With Antisense Oligonucleotides*. Lloyd Tillman, Isis Pharmaceuticals, Inc., Carlsbad, California, USA

1230h **Closing**: Concluding Remarks, Elizabeth Vadas, President, CSPS

Programme Committee

Elizabeth Vadas, Ph.D., INSCITECH Inc., Québec, **(Chair)**
William Casley, Ph.D., Health Canada, Ontario
Fakhreddin (Mo) Jamali, Ph.D., University of Alberta, Alberta
Lakshmi Kotra, Ph.D., University of Toronto, Ontario
Elizabeth Kwong, Ph.D., Merck Frosst Canada & Co., Québec
Gordon McKay, Ph.D., Pharmalytics Inc., Saskatchewan
Jasmine Prucha, Gattefossé Canada Inc., Ontario
Amyn Sayani, Ph.D., GlaxoSmithKline, Inc., Ontario
Jacques Turgeon, Ph.D., University of Montréal, Québec
Kishor M. Wasan, Ph.D., University of British Columbia, B.C.
Pollen K.F. Yeung, Ph.D., Dalhousie University, Nova Scotia

Contents

Eric Masson
President of CSPS

After graduating in Pharmacy from Université Laval, he obtained a Pharm.D. from SUNY Buffalo, then completed a fellowship in clinical pharmacology from St. Jude Children's Research Hospital, Memphis TN. Dr Masson was assistant professor in Pharmacy at Université Laval before joining Anapharm as Scientific Director in 1998. He has 10 years of experience in clinical research, particularly Phase I, and bioequivalence studies. He is currently Vice-President of Scientific and Regulatory Affairs at Anapharm-member of SFBC International. Dr Masson still maintains relationship with academia, lecturing courses to graduate students in drug development, and as external reviewers for Ph.D. thesis. His research interests include pharmacokinetics and pharmacodynamics, and trial simulations. Éric is also President of the Canadian Society of Pharmaceutical Scientists. He has presented in several international conference authored several scientific papers.

Elizabeth Vadas
Chair of the Scientific Meeting

Elizabeth B. Vadas received her Ph.D. in Physical Chemistry from McGill University in Montreal. She obtained her undergraduate degree in colloid and surface chemistry in Budapest, Hungary. Following postdoctoral training, she joined Merck Frosst, the Canadian subsidiary of Merck & Co. in 1980 as a senior research scientist in the department of Pharmaceutical Research and Development. Over the years, she has been involved in the formulation development of many new chemical entities discovered at the Merck Frosst Centre for Therapeutic Research while taking on increasing management responsibilities. Most recently Dr. Vadas was Executive Director of Pharmaceutical Research and Development; a department which has grown from 18 to over 80 scientists in 10 years under Dr. Vadas' leadership. Most notable of her scientific leadership accomplishments is the establishment of very close collaboration with the discovery groups in basic research resulting in the rapid selection of viable development candidates in the last decade. Notable technical accomplishments are the product development efforts of the department supporting the leukotriene and Cox-2 programs leading to worldwide regulatory approval of SINGULAIR®, Merck's new asthma therapy and VIOXX® in the Cox-2 class for the treatment of pain, osteoarthritis and rheumatoid arthritis. Her department also has developed ARCOXIA™, Merck's second coxib already approved in the EU and in South America. In mid-2002, Dr. Vadas has decided to take early retirement to establish her own consulting company InSciTech Inc., a company providing integrated solutions for scientific and technical problems faced by life science companies in the compound selection and product development areas. Currently she works with several drug discovery companies both in the US and Canada. Dr. Vadas' main scientific interests are in the area of pharmaceutics, particularly in solid-state chemistry and physics, drug excipient interactions and in drug delivery systems including rapid dissolving oral formulations and aerosols. She is an Adjunct Professor of Pharmaceutics at the Faculty of Pharmacy, University of Montreal. She also has lectured and published widely.

Merck Company Foundation Undergraduate Summer Student Award Recipients and Poster Competition

Kishor M. Wasan, National Director, Faculty of Pharmaceutical Sciences, University of B.C.

In order to encourage pharmacy students to go into research, funding in the form of research fellowships was established to give students an opportunity to work over the summer in research laboratories in all AFPC accredited Pharmacy Schools across Canada. The criteria of selection was based on academic excellence (i.e. grades), letters of reference and evidence of service to the faculty. Selection of the winners was determined by March 1st and all the winners and supervisors' names and a summary of their research project were sent to Merck Company Foundation.

Name of Recipient	Supervisor	Title of Project

Catherine Cheung
3rd Year Pharmacy, Dr. Thomas Chang, Interaction between Natural Health Products and Drug Metabolizing Enzymes

Frederic Normand
2nd Year Pharmacy, Dr. Patrice Hildgen, Étude des propriétés d'un vecteur actif nanoparticulaire pour le traitement du cancer par inhibition de l'angiogénèse

Cindy Lu
2nd Year Pharmacy, Dr. Ayman El-Kadi, The role of inflammation and ligand-activated nuclear receptor in the regulation of aryl hydrocarbon receptor-regulated genes

Daryl Fediuk
3rd Year Pharmacy, Dr. Xiaochen Gu, Effects of Temperature and Moisture content on Transdermal Absorption of Sunscreen/DEET products

John-Michael Gamble
1st Year Pharmacy, Dr. Fakhreddin Jamali, Pharmacokinetics of glucosamine: Site of metabolism in the rat

Rene Doucet
1st Year Pharmacy, Dr. David Jakeman, Synthetic Chemistry/Enzyme Cloning

Francesca Ting-Yan Cheung
2nd Year Pharmacy, Dr. Peter O'Brien, Molecular Cytotoxic Mechanisms of Drugs

Kimberly Ann Hogh
3rd Year Pharmacy, Dr. Adil Nazarali, The role of Hoxa2 Gene in Multiple Sclerosis

Jessica Fortin
2nd Year Pharmacy, Dr. Rene C. Gaudreault, In vivo and In vitro evaluation of the soft alkylating agents "C"loro Ethyl Ureas" (CEU) as anticancer drugs

Kishor Wasan
National Director of Canadian Undergraduate Pharmacy Student Research Program, Associate Professor & Chair of Pharmaceutics, University of British Columbia, Vancouver, Canada

Dr. Kishor M. Wasan is an Associate Professor & Chair of Pharmaceutics and National Director of the Canadian Summer Student Research Program at the University of British Columbia in Vancouver, BC, Canada since February 1995. He received his B.Sc in Pharmacy from the University of Texas at Austin in 1985 and his Ph.D. in Cellular and Molecular Pharmacology at the University of Texas Graduate School of Biomedical Sciences/Medical School at Houston in 1993. He completed a post-doctoral fellowship in Lipoprotein Biochemistry in the Department of Cell Biology at the Cleveland Clinic Foundation Research Institute. In the 7.5 years that Dr. Wasan has been an independent researcher at UBC, he has published over 85 peer-review articles and 125 abstracts in the area of lipid-based drug delivery and lipoprotein-hydrophobic drug interactions. Dr. Wasan has received over $4 million dollars of extramural funding and has currently trained 8 graduate students. Dr. Wasan was one of the recipients of the 1993 AAPS Graduate Student Awards for Excellence in Graduate Research in Drug Delivery and Pharmaceutical Technologies and recently was awarded the 2001 AAPS New Investigator Award/Grant in Pharmaceutics and Pharmaceutics Technologies sponsored by Pfizer Central Research and the 2002 Association of Faculties of Pharmacy of Canada AstraZeneca New Investigator Research Award. In January 2001, with funding from the Merck Company Foundation, Dr. Wasan established a Canadian Summer Student Research Program for Undergraduate Pharmacy Students. Furthermore, Dr. Wasan received the 2001/2002 University of British Columbia Killam Teaching Prize in Pharmaceutical Sciences. Dr. Wasan is the Associate Editor of the Journal of Pharmacy and Pharmaceutical Sciences and is on the editorial board of several other peer-reviewed journals. In addition, Dr. Wasan is a member of the Canadian Institutes of Health Research Pharmaceutical Sciences Grant Review Committee (Study Section). Currently Dr. Wasan's research is supported by several grants from The Canadian Institutes of Health Research, several pharmaceutical companies and the National Cancer Institute of Canada-Clinical Trials Group.

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SESSION 1

Impact Of Rational Drug Design On Pharmaceutical Development

Lakshmi Kotra
University of Toronto, The Leslie Dan Faculty of Pharmacy and Department of Chemistry, Molecular Design and Information Technology (MDIT) Center, Toronto, Ontario, Canada

Lakshmi Kotra is an assistant professor at the Faculty of Pharmacy and is cross-appointed to the Department of Chemistry at the University of Toronto. Dr. Kotra is the Director and a founding Executive Committee member of the Molecular Design and Information Technology Center at the University of Toronto. Dr. Kotra received his Ph.D. from the University of Georgia in 1997 and received postdoctoral training at Wayne State University before accepting the faculty position at the University of Toronto in 2000. Dr. Kotra is a recipient of Rx&D Health Research Foundation Research Career award (2001-06). Dr. Kotra serves on three editorial boards, as well as on several national review committees/ scientific program committees. Dr. Kotra's research interests focus in the areas of drug design, medicinal chemistry, understanding protein/nucleic acid-small molecule interactions and structure-based chemogenomics. Dr. Kotra's research program currently includes the design of novel mechanism-based strategies against proteases, discovery of novel immunomodulators, design of ligands against insulin receptor, design of ODCase inhibitors as potential antiviral drugs and design of target-based anticancer drugs.

Gordon McKay
PharmaLytics Inc., Saskatoon, Saskatchewan, Canada

Dr. Gordon McKay received his BSc. and Ph.D. degrees in Biochemistry from the University of Saskatchewan. He completed a postdoctoral position in the College of Pharmacy working with Dr. Kamal Midha and then went on to become a principal investigator in the research group supported by a Program Grant from the MRC. The research program is aimed at investigating the efficacy of antipsychotic drugs. Over the 11 years of support for this program, he has published in excess of 150 original manuscripts and numerous book chapters. He was awarded Fellowship in AAPS in 1994 for his contributions to analytical biochemistry especially as it relates to both biological based and chemical based methods of analysis and in particular mass spectrometry and allied techniques. He is a full professor of Pharmacy on leave of absence in order to establish a new corporate research institute called Pharmalytics Inc. in the University based research park. The mission of Pharmalytics is to engage in research and training in all areas of drug and drug product discovery and development especially as it relates to bioanalyses, biopharmaceutics, drug delivery and drug regulatory science; such that those pursuits lead to 1) safe, efficacious and high quality pharmaceuticals and 2) trained scientists and technologists for continuation of these pursuits.

Impact Of Targeted Libraries In Drug Discovery

Michael Bös, Chemistry Department, Boehringer Ingelheim (Canada) Ltd., Research & Development, Laval, Québec, Canada

Combinatorial Chemistry has become an indispensable tool in drug discovery. In the early days the main features of combichem screening libraries consisted in large numbers of compounds and simple chemical transformations for their production. Over the years the methodologies of synthesis have become more sophisticated, but the focus remained on the numbers of compounds accessible in a short period of time. However, it has been realized that this increase did not automatically translate into an increase in good chemical leads and that the hit rates from screening general unbiased libraries are substantially lower than hit rates from traditional medicinal chemistry compound libraries. Therefore, combinatorial chemistry has been used in recent years in a more directed sense for lead generation namely for the synthesis of so-called targeted compound libraries. The production of such biased libraries requires certain information about the therapeutic target and involves, depending on the nature of this information, medicinal chemistry as well as structural chemistry. This approach can also be used for the synthesis of small directed libraries for lead optimization purposes. The impact of such targeted libraries in the development of potent inhibitors will be discussed for the HCV NS3 serine protease. This enzyme is essential for replication of the Hepatitis C virus and inhibitors of the protease are potential therapeutic agents of HCV infection.

Michael Bös
Director, Chemistry Department, Boehringer Ingelheim (Canada) Ltd., Research & Development, Laval, Québec, Canada

Dr. Michael Bös is the director of chemistry at Boehringer Ingelheim (Canada) Ltd., Laval, QC. He received his Master's degree in pharmacy (1979) and his Ph.D. in medicinal chemistry (1981) from the University of Vienna, Austria. After postdoctoral studies at the ETH Zurich with Prof. D. Seebach (1982) and at CSU in Fort Collins, USA with Prof. A.I. Meyers (1983-1985) he joined F. Hoffmann-La Roche in Basel, Switzerland, as a medicinal chemist in 1986. At Roche, Dr. Bös was involved in a number of projects targeting diseases of the CNS such as depression, anxiety, obsessive compulsive disorder and Alzheimer's disease. As an exchange scientist at Roche in Nutley, New Jersey (1989-1990), he worked on peptidomimetics of Thyrotropine Releasing Hormone, where he applied computer assisted modeling for the design of new non-peptidic scaffolds. In 1999 he moved to Canada to take up his current position. Dr. Bös teaches medicinal chemistry at the Johann Wolfgang Goethe University, Frankfurt, in Germany.

Impact of NMR/Structural Biology in Drug Discovery

Stephen W. Fesik, Divisional Vice President, Cancer Research, Abbott Labs, Chicago, Illinois, USA

NMR spectroscopy is a useful tool in drug research. From the NMR structures of proteins and protein/ligand complexes, information can be obtained on a protein's mechanism of action, whether a protein is a suitable drug target, and the structural details of how natural ligands bind to the protein. This information is useful for the target identification/validation stage of the drug discovery process. From NMR-based screening, small molecules that bind to a protein target can be found to aid in lead identification. In addition, NMR can be used to determine how these small molecules bind to the protein, which is useful for lead optimization. In this presentation, the utility of NMR spectroscopy in drug research will be illustrated in the discovery of potent matrix metalloprotease inhibitors for the treatment of cancer and small molecules that bind to Bcl-2/Bcl-XL and promote apoptosis in tumor cells.

Stephen Fesik
Divisional Vice President, Cancer Research, Abbott Laboratories, Chicago, Illinois, USA

Stephen Fesik obtained his Ph.D. in Medicinal Chemistry from the University of Connecticut in 1981 and was a postdoctoral associate at Yale University in the Department of Molecular Biophysics and Biochemistry from 1981 to 1983. After his postdoctoral work, he joined Abbott Laboratories. At Abbott, he developed several new NMR methods that are widely used today. He contributed to the development and application of isotope-edited NMR experiments for studying molecular complexes, first described heteronuclear three-dimensional NMR spectroscopy, and pioneered NMR experiments that utilized ^{13}C-^{13}C magnetization transfer by isotropic mixing. He also determined the three-dimensional structures of several proteins and protein/ligand complexes. In addition to these structural studies, he developed a method for drug discovery called SAR by NMR and applied this method to identify and optimize ligands for binding to many protein drug targets. Dr. Fesik has published 198 papers, trained 27 postdoctoral fellows, has been a reviewer for the NIH Biophysical Chemistry Study Section, and has served as a member of the Editorial Boards of the Journal of Medicinal Chemistry, Journal of Biomolecular NMR, Biophysical Journal, and Molecular Cell. In addition, he is a member of the Highlights Advisory Panel for Nature Reviews Cancer, the Keystone Advisory Board, and the Scientific Advisory Committee for the Cyprus Conference of New Methods in Drug Research. He has obtained several awards, including the Chairman's Award (1996) and Outstanding Researcher of the Year Award (1997) at Abbott Laboratories, the Servier Lectures Award (1998) from the University of Montreal, and the ASBMB-Fritz Lipmann Award (1999). He is currently Divisional Vice President of Cancer Research at Abbott Laboratories where he leads a group responsible for discovering new drugs to treat cancer.

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Physicochemical Properties in Drug Design

Christopher A. Lipinski, Adjunct Senior Research Fellow, Pfizer Global Research and Development, Groton Laboratories, Groton, Connecticut, USA

Poor aqueous solubility is the single largest physicochemical problem hindering oral drug absorption and lengthening drug discovery time in the current HTS / combinatorial chemistry era. Current experimental early discovery stage solubility screens differ markedly from traditional thermodynamic solubility assays. The solubility ranking of collections of chemical compounds can be estimated either from data mining, e.g. using the "rule of five" or from internal experimental solubility measurements or from calculations from among the many commercially available solubility programs. Poor aqueous solubility also adversely affects data quality in Caco-2 permeability screens. The problem of poor physicochemical properties is not solely a technical issue that can be solved by better experimental assays or computational predictions. Effective communication between pharmaceutical sciences and medicinal chemists is essential. In this regard, it is critical to realize that chemists think differently than pharmaceutical scientists. In dealing with chemists it is important to appeal to the chemists highly developed pattern recognition skills and to avoid the use of mathematical equations as much as possible. In terms of technology resource allocation there is no excuse for not understanding the likely solubility rankings of collections of compounds. Early discovery technology teams screening for poor ADME properties can be one of the pharmaceutical scientist's best allies in preventing the progression of seriously flawed compounds into clinical candidate nomination.

Christopher Lipinski
Adjunct Senior Research Fellow, Pfizer Global Research and Development, Groton Laboratories, Groton, Connecticut, USA

Dr. Lipinski retired from the position of Senior Research Fellow in the Exploratory Medicinal Sciences Department at the Pfizer Global Research and Development Groton Laboratories in June 2002. He received a B.Sc. degree in chemistry from San Francisco State College in 1965 and a Ph.D. in 1968 in physical organic chemistry from the University of California, Berkeley. He joined Pfizer in 1970 following a National Institutes of General Medical Sciences Postdoctoral Fellowship at the California Institute of Technology. At Pfizer from 1970 to 1990, he supervised medicinal chemistry drug discovery laboratories discovering multiple gastrointestinal and diabetic clinical candidates. In this process, he became interested in the design of bioisosteres and in drug physical chemical properties and quantitative structure activity relationships, especially as they related to problems of oral activity. In 1990, he established a highly automated laboratory combining computations and experimental physical property measurements. Experimentally, his laboratory provided experimental solubility measurements on medicinal compounds synthesized at the Pfizer Groton site. Computationally he champions a very pragmatic, chemistry end user oriented, approach to the problem of oral activity improvement. He is a member of the Medicinal Chemistry section of the American Chemical Society, the American Association of Pharmaceutical Sciences, the Society for Biomolecular Screening and the European Federation of Pharmaceutical Sciences. He serves on scientific advisory boards for: the Global Alliance for TB Drug Development, ASDI II, Advanced Chemistry Development Labs and the Matrical company. He is a member of the editorial board of the journal of Pharmaceutical Sciences. Since 1984, he has been an adjunct faculty member at Connecticut College in New London CT, and has over 160 publications and invited presentations and 17 issued US patents.

The Critical Role of Informatics In Exploratory Drug Development

Mark L. Powell, Vice-President, Analytical Research and Development, Bristol-Myers Squibb Company, Princeton, New Jersey, USA

The dramatic increase in combinatorial chemistry and high throughput screening activities has revolutionized drug discovery. The resultant increase in available data has underscored the critical role of informatics. This critical role has now reached Exploratory Drug Development (lead optimization to Phase IIa of clinical development) as pharmaceutical companies continually search for ways to shorten development timelines and increase the quality of their compounds. Emerging registration requirements are also requiring changes in how certain types of data are archived and submitted. Bristol-Myers Squibb has taken a fully integrated approach in looking at informatics support across all of exploratory drug development. This major initiative encompasses the departments of clinical pharmacology, drug metabolism and pharmacokinetics, drug safety and evaluation and regulatory affairs. Industry benchmarking led to the refinement of the initiative scope and eventual launch of 13 different projects including direct data capture and electronic data capture of early clinical data, time-response image capture, archive and transmission of data. The standardization of pharmacokinetic data and evaluation was also included – just to name a few. The initiative took approximately 3 months to conceptualize and make recommendations and is now in its third year of implementation. This integrated approach is leading to a smoother integration of new software solutions and business practices as exploratory drug development continues to evolve from its historical approaches.

Mark Powell
Vice-President, Analytical Research and Development, Bristol-Myers Squibb Company, Princeton, New Jersey, USA

Dr. Powell is currently the Vice President of Analytical Research and Development at Bristol-Myers Squibb. He is responsible for all analytical activities, which includes all of BMS's compounds in Global Pharmaceutical Development, on 5 research campuses in the U.S. and Canada. Dr. Powell obtained his Ph.D. from the University of Washington in 1980 and has worked in the pharmaceutical industry for over 20 years. Dr. Powell's professional experiences have encompassed all aspects of analytics, bioanalytics, drug metabolism and pharmacokinetics research and development, from discovery lead compound optimization and selection through formulation evaluation, dosage form selection, optimization and production support, to preclinical and clinical drug development (including the clinical trial process) as he has assumed increasing responsibilities throughout his career. In addition to his functional responsibilities at BMS, he is the global Exploratory Development Project leader for Diabetes and the Co-Leader of a company-wide informatics initiative called Endeavor. This initiative focuses on the development and implementation of innovative informatics-based solutions in the areas of Exploratory Drug Development (Candidate Lead Optimization, Drug Safety Evaluation, Drug Metabolism and Pharmacokinetics, early Clinical Development, etc.). Dr. Powell has authored and co-authored more than 125 scientific articles and presentations that have been made at the local, national and international level, as well as given numerous invited talks. He has chaired many scientific discussion panels at both national and international meetings and has chaired an international meeting.

SESSION 2

Pharmacokinetics,
Pre-Clinical ADME, Toxicology

Elizabeth Kwong
Director, Department of Pharmaceutical Research and Development, Centre for Therapeutic Research, Meck Frosst Canada & Co., Kirkland, Quebec, Canada

Dr. Elizabeth C. Kwong is a Director at Merck Frosst Canada & Co. Centre for Therapeutic Research, Department of Pharmaceutical Research and Development (PR&D). Dr Kwong leads the analytical division in PR&D. She is currently appointed Associate Professor in the Dept. of Pharmaceutics at the University of Montreal. She received her B.S. in Pharmacy (1980) and Ph.D. degree in Pharmaceutical Chemistry (1980-1984) from the University of British Columbia, Canada. Dr. Kwong completed a postdoctoral fellowship in Pharmaceutics at the School of Pharmacy, University of Washington in Seattle (1984-1986). She then joined Cantest Laboratories, a contract facility in Vancouver, B.C. as a Research Scientist prior to taking a Senior Research Chemist position at Merck Frosst in 1988. Her research experience includes development of analytical methods for the sensitive quantitation of drugs in biological fluids and analytical methods to support formulation development.

Kishor Wasan
National Director of Canadian Undergraduate Pharmacy Student Research Program, Associate Professor & Chair of Pharmaceutics, University of British Columbia, Vancouver, Canada

Dr. Kishor M. Wasan is an Associate Professor & Chair of Pharmaceutics and National Director of the Canadian Summer Student Research Program at the University of British Columbia in Vancouver, BC, Canada since February 1995. He received his B.Sc in Pharmacy from the University of Texas at Austin in 1985 and his Ph.D. in Cellular and Molecular Pharmacology at the University of Texas Graduate School of Biomedical Sciences/Medical School at Houston in 1993. He completed a post-doctoral fellowship in Lipoprotein Biochemistry in the Department of Cell Biology at the Cleveland Clinic Foundation Research Institute. In the 7.5 years that Dr. Wasan has been an independent researcher at UBC, he has published over 85 peer-review articles and 125 abstracts in the area of lipid-based drug delivery and lipoprotein-hydrophobic drug interactions. Dr. Wasan has received over $4 million dollars of extramural funding and has currently trained 8 graduate students. Dr. Wasan was one of the recipients of the 1993 AAPS Graduate Student Awards for Excellence in Graduate Research in Drug Delivery and Pharmaceutical Technologies and recently was awarded the 2001 AAPS New Investigator Award/Grant in Pharmaceutics and Pharmaceutics Technologies sponsored by Pfizer Central Research and the 2002 Association of Faculties of Pharmacy of Canada AstraZeneca New Investigator Research Award. In January 2001, with funding from the Merck Company Foundation, Dr. Wasan established a Canadian Summer Student Research Program for Undergraduate Pharmacy Students. Furthermore, Dr. Wasan received the 2001/2002 University of British Columbia Killam Teaching Prize in Pharmaceutical Sciences. Dr. Wasan is the Associate Editor of the Journal of Pharmacy and Pharmaceutical Sciences and is on the editorial board of several other peer-reviewed journals. In addition, Dr. Wasan is a member of the Canadian Institutes of Health Research Pharmaceutical Sciences Grant Review Committee (Study Section). Currently Dr. Wasan's research is supported by several grants from The Canadian Institutes of Health Research, several pharmaceutical companies and the National Cancer Institute of Canada-Clinical Trials Group.

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Modelling of intestinal drug absorption and bioavailability

K. Sandy Pang, Professor, Pharmacy and Pharmacology, Faculties of Pharmacy and Medicine, University of Toronto, Toronto, Ontario, Canada

In addition to the liver, the intestine is an important tissue that regulates the extent of oral absorption of drugs because of first-pass metabolism. Intestinal absorption is affected by both drug characteristics and the physiology of the gastrointestinal tract (GIT). Absorption is mediated by intestinal transporters for organic anions and cations, and by passive absorption for lipophilic drugs due to the large surface area because of the villi and microvilli of the small intestine. However, efflux by the P-glycoprotein (Pgp) or multidrug-resistance associated protein 2 (MRP2) mitigates the cumulative amount absorbed. Intestinal enzymes, albeit present at lower levels to those in liver, mediate both phase I and phase II metabolism. Although much work exists for the characterization of intestinal drug uptake/efflux and metabolism with the various in vitro systems, the quantitative interpretation of data in vivo remains scarce. Modeling efforts have centered on developing physiologically-based models to view intestinal transporters for absorption and efflux in concert with drug metabolizing enzymes. For example, a traditional, physiologically-based model (TM) was used to describe the dynamic interplay of the processes. But due to the greater intestinal metabolism/ extraction noted in animal and human studies with oral or luminal vs. "systemic" dosing, the Segregated Flow Model (SFM) that incorporates separate intestinal tissues and flows to a non-absorptive and an absorptive, outermost layer (enterocytes), was utilized to describe the so-called "route-dependent" intestinal metabolism. These models have been further extended to include various duodenum, jejunum and ileum segments. The expanded Segmental Segregated Flow Model (SSFM) and the Segmental, Traditional Model (STM) view the intestine as three segments of equal lengths receiving equal flows in order to accommodate heterogeneities in transporter and metabolic functions. Exploration of the properties of the models reveals that the heterogeneity in absorptive, exsorptive or metabolic functions further impacts on intestinal drug clearance, bioavailability and metabolite formation.

Sandy Pang
Professor, Pharmacy and Pharmacology, Faculties of Pharmacy and Medicine, University of Toronto, Toronto, Ontario, Canada

K. Sandy Pang, Ph.D., is Professor of Pharmacy and Pharmacology, Faculties of Pharmacy and Medicine at the University of Toronto. She was the recipient of the NIH Research Career Development Award, Faculty Development award from MRC Canada, the McNeil Award from the Faculties of Pharmacies in Canada, and the Research Achievement Award in Pharmacokinetics, Pharmacodynamics and Drug Metabolism from the American Association of Pharmaceutical Scientists (AAPS). Her research is aimed towards a mechanistic-based understanding of drugs and metabolite processing within the liver, the intestine, and kidney via integration of theory and experimentation. This is achieved through the development of formulations of physiologically relevant processes and validation by experimental approaches. Her work emphasizes the presence of sequential removal of formed metabolites *in situ* the eliminating organ. She stressed that much difference existed in the disposal of formed *vs*. preformed metabolites because of transmembrane barriers, enzyme heterogeneity, enzymatic coupling, and owing to the kinetics on successive formation of metabolites. She introduced perfusion methods such as prograde-retrograde and hepatic arterial-portal venous and hepatic arterial-hepatic venous perfusions to probe enzyme/transport activity. She adopted an integrated approach to examine transporter and metabolic functions in expression (cDNA) systems, zonal cells, and the multiple indicator dilution technique to map liver transporter and enzyme activities for modeling of metabolic events. Her recent focus is examination of heterogeneity in intestinal transport and metabolism in absorption. Recognition of route-dependent metabolism by the intestinal tissue, notably metabolism upon oral and not systemic dosing of drug had lead her to develop the physiologically based, segregated flow (SFM) and segmental, segregated flow (SSFM) models.

Pharmacokinetic studies on the developing blood brain barrier in sheep using diphenhydramine

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

The function of the blood brain barrier was investigated in chronically instrumented fetuses (100 and 120 d gestation), newborn lambs (10 and 30 d), and adult sheep by examining the uptake of the H_1-receptor antagonist diphenhydramine (DPHM) into the CNS [extracellular fluid (ECF) and cerebrospinal fluid (CSF)], using microdialysis. Probes were implanted into the lateral ventricle and parietal lobe of the cerebral cortex for CSF and ECF sampling. Two different studies were used. The first involved *i.v.* administration of DPHM at five infusion rates, with each step lasting 7 h. In all age groups, CSF and ECF concentrations were very similar, suggesting passive diffusion of DPHM between these two compartments. The brain-to-plasma concentration ratios were 3 or higher in all groups, implying the existence of a transport process for DPHM into the brain. The AUC_{brain}/AUC_{plasma} ratios decreased with age, suggesting maturation of barrier properties and that DPHM was more efficiently removed from the brain as age increased. In the second study, DPHM was infused for 8 h and propranolol was co-infused from 4 – 8 h. While DPHM Cl_T and extent of plasma protein binding remained unchanged, the AUC_{brain}/AUC_{plasma} ratios increased in all groups with the greatest increase in the adult sheep. The observed increase in the ratios may be due to the inhibitory effects of propranolol on a transporter-mediated efflux mechanism for DPHM brain elimination, the activity of which appears to increase with development. These two studies suggest the existence of two distinct transporter-mediated mechanisms for the influx and efflux of DPHM into and out of the brain. A definitive answer as to the identity of these transporters will require further investigation.

Wayne Riggs
Professor, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada

Dr. Riggs is a professor at the Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada. He received his B. Sc. in pharmacy in 1971 from the University of British Columbia (UBC). After working in pharmacy practice for eight years, he returned to UBC to obtain a M. Sc. (1982) in biopharmaceutics, and following two additional years in practice his Ph. D. (1989) in pharmacokinetics. He joined the faculty at UBC in 1989. His research interests include pharmacokinetic studies of placental drug transfer and fetal drug effects, uptake of drugs into the central nervous system and the effects of individual genetic variation on drug pharmacokinetics and response. He has published more than 100 refereed articles and abstracts, and has been an invited speaker at a number of conferences. He has trained 2 M.Sc. and 8 Ph.D. students. Dr. Riggs teaches pharmacokinetics and mass spectrometric analysis at the undergraduate and graduate levels and is actively involved in pharmacy curriculum development and assessment. He has received the University of British Columbia Killam Teaching Prize in recognition of his teaching achievements. Dr. Riggs has served as a pharmacokinetics consultant to several biotech and pharmaceutical companies and is an ad-hoc member of the Health Canada Therapeutic Products Directorate's Expert Advisory Committee on Bioavailability and Bioequivalence. He is currently co-chair of the organizing committee for the 7th International meeting of the International Society for the Study of Xenobiotics (ISSX) being held in Vancouver, British Columbia, Canada, August 29 – September 2, 2004. (http//www.ubcpharmacy.org)

Nonclinical Toxicology/Safety Studies to Support Initiation and Continuation of Clinical Trials

Glenn R. Washer, Director, Toxicology & Safety Assessment, Theratechnologies Inc., Montreal, Quebec, Canada

The general concepts and designs of Phase I, II and III clinical trials are widely known among members of the pharmaceutical industry and results from clinical trials draw great attention from all quarters. However, before human trials can be initiated, international regulatory bodies require a host of in-depth assessments of toxicity and safety in several species as part of a nonclinical development program. The information gained from such work can have a material impact upon the design and conduct of the clinical trials that follow and, when critically designed and evaluated, these animal studies can help reduce the risk of failure in human studies. This talk will give an overview of the international regulations guiding the conduct of nonclinical studies, the basic designs and objectives of toxicology, general toxicology, safety pharmacology, reproductive toxicology and carcinogenicity studies, and relate these studies to the various stages of clinical development.

Glenn Washer
Director, Toxicology & Safety Assessment,
Theratechnologies Inc., Montreal, Quebec, Canada

Glenn Washer is the Director of Toxicology and Safety Assessment at Theratechnologies Inc., a leading peptide-based biopharmaceutical company targeting endocrine and metabolic disorders, located in Montreal, Canada. He is responsible for the design, oversight and conduct of all preclinical safety programs necessary to permit the initiation and continuation of the company's clinical trials. Prior to joining Theratechnologies, Mr. Washer spent 19 years at CTBR, a major preclinical contract research laboratory, where he was the Scientific Director of Infusion, Pharmacology and Neurotoxicology from 1994 to 2002. In this role he managed a group of 40 scientists and technicians, and was responsible for providing senior-level input into safety program design and evaluation on hundreds of pharmaceutical and biopharmaceutical products. From 1989 to 1994, Mr. Washer served as a study director on over 200 pharmaceutical toxicology studies, and from 1983 to 1989, he gained extensive technical expertise in pathology and toxicology. Mr. Washer is an industry-recognized expert in the conduct of infusion/injection toxicology studies and has published over 30 abstracts on this and related topics. In addition to Mr. Washer's involvement as a CSPS lecturer, he has lectured on various topics for the Pharmaceutical Education and Research Institute (PERI), the Society of Toxicology, McGill University and Université de Montreal, and was organizer and lecturer of several continuing education courses in toxicology and preclinical development. Mr. Washer is a graduate of McGill University with a degree in Physiology and is certified in General Toxicology by the American Board of Toxicology.

Prediction of Plasma and Tissues Kinetics of Drugs in Animals and Human using In Silico and In Vitro Data as Sole Input in Generic Physiologically-Based Pharmacokinetics (PBPK) Models.

Patrick Poulin, Senior Scientist, Biomodeling & Simulation Group, Non Clinical-Drug Safety, F. Hoffmann-La Roche Ltd., Pharma Division, Basel-Stadt, Switzerland

PBPK models can provide a framework to integrate the *in vitro* or predicted data on ADME available at all stages of drug development. In this context, PBPK models can be used as a simulation tool for providing predictions of plasma and tissue concentration-time profiles of novel drug candidates prior to any *in vivo* studies in animals and human. During the drug development process, the *in vivo* concentration-time profiles become available for each selected drug. The comparison of simulated with experimental data can provide mechanistic information whether the key ADME processes are covered or not in the models. In this sense, PBPK models can also be used as diagnostic tools to understand the *in vivo* data in a more mechanistic manner. The objective of the presentation will be to illustrate that generic PBPK model frameworks, which are useful simulation and diagnostic tools, can be developed prior to any *in vivo* studies. Absorption, tissue distribution and liver metabolism are the processes currently integrated into the generic PBPK models. The rate and extent of gastrointestinal absorption are estimated based upon *in vitro* solubility and permeability information by using commercially available software. Distribution is estimated with an in-house made software built from tissue composition-based equations into which physicochemical and in vitro data on plasma and lipid binding are used as sole input parameters. Furthermore, data on intrinsic clearance (CL_{int}) determined *in vitro* with hepatocytes were also incorporated in these models to estimate metabolic clearance under *in vivo* conditions. The above methodology will be illustrated with compound examples and compared with conventional allometric scaling approaches. An overall modeling strategy will also be presented. This new methodology opens opportunities for the use of generic PBPK models in drug discovery and development, which may potentially integrate additional *in vitro* data and information on other ADME processes. PBPK models could therefore potentially be used to speed up the selection and optimization of new drug candidates.

Patrick Poulin
Senior Scientist, Biomodeling & Simulation Group, Non Clinical-Drug Safety, F. Hoffmann-La Roche Ltd., Pharma Division, Basel-Stadt, Switzerland

Patrick, who is a Canadian citizen, has obtained a BScA degree in Food Sciences and Technology at the Université Laval in Quebec City, and a PhD degree in Toxicology at the University of Montréal. Following his academic experiences, he made a postdoctoral fellowship in pharmaceutical sciences at the company F. Hoffmann-La Roche located in Basel, Switzerland. Patrick is currently a senior scientist and his main responsibility consists of making the Physiologically Based Pharmacokinetic (PBPK) models more useful for drug discovery and pre-clinical. These models are used to help the selection of clinical candidates based upon more rationale and effective screening efforts of PK-PD relationships. An important part of Patrick's work is also to perform advanced studies on the tissue distribution of drugs, which is an essential process for PBPK modeling. Consequently, he develops mechanism-based prediction tools for the volume of distribution and tissue:plasma partition coefficients. Beside his work at Hoffmann-La Roche, Patrick contributes to several review activities in different journals. He is also a co-author of two general book chapters and several papers on the topic of PBPK modeling. Furthermore, he is a member of the consortium on Applied Pharmacokinetic Researches, which regroups the Manchester University and six large pharmaceutical companies.

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SESSION 3

Biomarkers As Surrogate Endpoints In Pre-Cliical And Clinical Development

Pollen K.F. Yeung
Professor and Director, Pharmacokinetics and Metabolism Laboratory, College of Pharmacy, Dalhousie University, Halifax, Nova Scotia, Canada

Pollen Yeung is currently Professor and Director of the Pharmacokinetics and Metabolism Laboratory, College of Pharmacy, Dalhousie University, Halifax, Nova Scotia, Canada. He obtained his undergraduate pharmacy degree (B.Sc. Pharm. 1979) and M.Sc. (1982) from the University of Manitoba, Winnipeg, Manitoba, and Ph.D. in pharmaceutical chemistry from the University of Saskatchewan (1986), Saskatoon, Saskatchewan. He joined the College of Pharmacy, Dalhousie University in Halifax, as an Assistant Professor in 1985, and was promoted to Full Professor in 1996. He is also crossed appointed with the Department of Medicine at Dalhousie University and QEII Health Sciences Centre. His research interests are in the area of pharmacokinetics, metabolism and pharmacogenomics of cardiovascular drugs, and their effects on hemodynamic and neurohormone regulation, which has received support from both government and pharmaceutical industry. He has served as a referee and consultant for many academic and professional organizations, pharmaceutical industry, government and granting councils. He is currently a member of the Board for BioNova that is an industry association promoting biotechnology and life sciences industry in Nova Scotia and Atlantic Canada, and a member of the executive council for the Canadian Society of Pharmaceutical Scientists.

William Casley
Research Scientist, Centre for Biologics Research, Biologics and Genetic Therapies Directorate, Health Canada, Sir F.G. Banting Research Centre, Tunney's Pasture, Ottawa, Ontario, Canada

Bill Casley is a research scientist in the Molecular Biology Division, at the Centre for Biologics Research in the Biologics and Genetic Therapies Directorate of Health Canada, in Ottawa. He joined Health Canada in 1992 and developed a research program in pharmacogenetics, in recognition of the increasing impact of this area on the discovery, development and marketing of new therapies. Dr. Casley's main research interest is the genetic complexity of drug metabolism, using genomic analysis in mice to identify novel determinants of pharmacogenetic variation. He is also involved in collaborative pharmacogenomics research with scientists in Canada and the United States involving aboriginal populations of the Canadian north. Dr. Casley is also active in the area of applied research to ensure the safety of blood -derived therapeutics. He was a member of the expert working group which drafted the General Method for Nucleic Acid Testing for the European Pharmacopoeia and is currently a member of the World Health Organization working group for the Standardization of Nucleic Acid Testing for Viral Contaminants in Blood and Blood Products. Dr. Casley also participates in several international collaborative studies on methodologies for viral screening.

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Current Drug Discovery Programs at Merck Frosst: Bridging Drug Discovery to the Clinic with Surrogate Markers

Kathleen Mary Metters, Vice-President, Centre for Therapeutic Research, Merck Frosst Canada & Co., Kirkland,Quebec, Canada

Kathleen Metters
Vice-President, Centre for Therapeutic Research, Merck Frosst Canada & Co., Kirkland,Quebec, Canada

Dr. Metters is currently Vice President & Site Head of the Merck Frosst Centre for Therapeutic Research located in Kirkland, Quebec. Merck Frosst is a Division of Merck Sharpe & Dohme Research Laboratories, located in Whitehouse Station, New Jersey. Kathleen graduated with a Bachelor's degree in biochemistry from the University of Manchester Institute for Science and Technology, and with a PhD. from the Imperial College of Science and Technology in London. After a post-doctoral fellowship in the Laboratoire de Physiologie Nerveuse at the CNRS, she came to Montréal for additional post-doctoral training at the Institut de Recherche Clinique de Montréal before joining Merck Frosst in 1988. Throughout her academic training Kathleen received many awards including the Fondation de France Scholarship, La Fondation Simone et Cino del ducca Scholarship and the C.N.R.S Post Rouge-Scholarship. From June 1999 to the present time, Kathleen has served as an Adjunct Professor in the Department of Pharmacology and Therapeutics, McGill University, Montreal. When Kathleen joined Merck Frosst she worked on the arachidonic acid cascade. Her efforts were instrumental to the success of both the leukotriene (Singulair®) and COX-2 (Vioxx®) inhibitor programs. For her role on the leukotriene antagonist program, she was awarded a Merck Divisional Scientific Award, and was one of the team of scientists named in the winning nomination for the Prix Galien Canada 2000, for excellence in innovative research. Kathleen's focus was next directed into the areas of prostaglandin research and her team focused on targets of the prostaglandins, and led an aggressive effort to characterize the full family of prostanoid receptors. The target that emerged from this analysis, having the greatest degree of preclinical validation, was the DP receptor, with allergic rhinitis as the disease condition. She has published over 50-refereed articles, filed 23 Patents and co-authored 12 review books and chapters; and has been an invited speaker at many international conferences. www.merckfrosstlab.ca

Integrating Preclinical And Clinical Evidence: Linking Drug Pharmacology And Empiricism

Terrence F. Blaschke, Professor of Medicine and of Molecular Pharmacology; Chief, Division of Clinical Pharmacology, Stanford University School of Medicine; Adjunct Professor of Biopharmaceutical Sciences, School of Pharmacy, University of California, San Francisco, California, USA

The traditional approach to drug development has relied heavily on empiricism, the practice of relying on observation and experiment founded on experience without the aid of science or theory. While empiricism is necessary, it is not sufficient as it does not establish pharmacologic causality and therefore has limited value for extrapolation to future patients because it does not establish that a drug's beneficial effect is due to the drug's pharmacological action. Given an empirical association in preclinical or clinical studies, it is necessary to establish causality by demonstrating pharmacological activity as the mechanism for the beneficial, and in some cases, toxic effects of the drug. This is done by designing studies that provide evidence supporting pharmacological activity. Examples of studies would be those that are designed to show that: Response correlates with (temporally varying) exposure; Causal path biomarkers change in a mechanistically compatible direction, rate, and temporal sequence. An intermediate step in investigating causal path biomarkers is the development of scientifically-based mechanistic or partially mechanistic models of drugs and diseases. An important hypothesis is that mechanistic PK/PD models with biomarkers in preclinical animal studies will predict biomarker responses in humans. To test this hypothesis requires a commitment to biomarker determinations and model- driven "methods studies" in humans. The challenges in this approach are identifying and qualifying causal path biomarkers, developing and qualifying mechanistic pharmacodynamics (PD) models, then using modeling and simulation to make decisions by quantifying the uncertainty around the decision. An example of models for the development of antiretroviral drugs will be presented.

Terrence Blaschke
Professor of Medicine and of Molecular Pharmacology; Chief, Division of Clinical Pharmacology, Stanford University School of Medicine; Adjunct Professor of Biopharmaceutical Sciences, School of Pharmacy, University of California, San Francisco, California, USA

Dr. Blaschke received his medical degree from Columbia University College of Physicians and Surgeons, and after residency training in Internal Medicine at UCLA Center for Health Sciences, he was a Clinical Associate in the Metabolism Branch at the National Institutes of Health. Following fellowship training in Clinical Pharmacology at the University of California, San Francisco, Dr. Blaschke joined the faculty at the Stanford University School of Medicine in 1974 where he is Professor of Medicine and Molecular Pharmacology and Chief of the Division of Clinical Pharmacology. In 1999 he received the Henry J. Kaiser Award for Outstanding Contributions to Medical Education. He is a past president of ASCPT and has also served as Chairman of the Scientific Program Committee, the Long Range Planning Committee, the Scientific Awards Committee and the ASCPT Liaison Committee for Clinical Pharmacology. In 2002, he received the Rawls-Palmer award from ASCPT for significant contributions to drug investigation that brings the efforts of modern drug research to the care of patients. He is a past Chair of the Generic Drugs Advisory Committee and is a Special Government Employee for the FDA. He has been a consultant to a number of pharmaceutical firms and was a director of Therapeutic Discovery Corporation and Crescendo Pharmaceuticals, both early-stage drug development companies. He chaired the Drug Utilization Review Panel of USP from 1995-2000. He serves on the Charter Science Board of the Center for Drug Development Science at Georgetown University Medical Center. In June 2002 he returned to the full time faculty after a leave of absence at Pharsight Corporation, in Mountain View, California. He was appointed as Associate Dean for Medical Student Advising at Stanford upon his return to Stanford. His research has been primarily clinical, with a focus on the clinical pharmacology of drugs used in patients with HIV infection, the effects of non-adherence on viral and clinical responses, and a second focus on cardiovascular pharmacology, with an emphasis on modeling exposure-response relationships. His involvement in clinical trials and with the pharmaceutical industry has lead to a strong interest in approaches to improve the drug development process.

Comprehensive Difference Mapping And Protein Identification In Biomarker Studies

Daniel Chelsky, Chief Scientific Officer, Caprion Pharmaceuticals, Montreal, Quebec, Canada

Much of the early work in proteomics has consisted of identifying the protein content of a biological sample. While this information can be important, many interesting insights come from observing differences between samples. These differences can, for example, be a function of drug treatment, disease state, or the stage of a process. For highly complex samples, such as human serum, cataloging the entire proteome may not be practical, or even possible, particularly for each sample analyzed. As a result, we have developed the ability to match highly complex, non-identical, samples and to quantify the differences between them. Applying this tool to mass spectrometry, thousands of peptide ions from normal and diseased individuals are compared. Those peptide ions that differ or are unique to one set of samples are fragmented by MS/MS. The sequence is obtained and matched to the parent protein using genomic information. This work is giving us, at minimum, biomarkers of disease. From the sequence information obtained, new insights into the disease may be possible as well. This approach is also being applied to other studies, including drug response in serum and tissues, as well as to the identification of immunotherapy targets in colon and lung cancer.

Daniel Chelsky
Chief Scientific Officer, Caprion Pharmaceuticals, Montreal, Quebec, Canada

Dr. Chelsky is the Chief Scientific Officer of Caprion Pharmaceuticals, a company using proteomics to discover new targets for disease intervention, mechanisms of drug action, and biomarkers of disease. Previously he served as President of BioSignal Inc., a subsidiary of PerkinElmer focused on G-protein coupled receptor related research and reagents. He has also held positions as Sr. Director of Biology at Pharmacopeia, Director of Drug Discovery at Onyx Pharmaceuticals, and Principal Investigator at DuPont Merck Pharmaceuticals. He received his Ph.D. at the University of Oregon and was an American Cancer Society fellow at the University of California, Berkeley.

Regulatory Perspective On Biomarkers As Surrogate Endpoints

Agnes V. Klein, Manager, Clinical Evaluation Division, Biologics and Radiopharmaceuticals Evaluation Centre, Biologics and Genetic Therapies Directorate, Ottawa, Ontario, Canada

Biomarkers have been used in diagnosis in medicine for some time. As examples, the changes in Alkaline Phosphatase have been used as one of the indicators of hepatic canalicular disease, while CPK had been a mainstay of diagnosis in coronary artery disease. Concerted efforts have been placed in the last decade or so to find "the marker for each disease", the "holy grail", the one biomarker that is specific for each disease and allows one to chart its progress or cure. This is true in two situations in particular: Cancer and Heart Disease. These two have yielded several opportunities to explore the value of biomarkers. In particular, in Cancer and since the discovery of the Carcinoembryonic antigen (CEA), researchers have tried to exploit and mine this and other markers as indicators of the effectiveness of different therapies and to forestall or predict recurrence or spread of disease well before it becomes clinically evident and thus more difficult to manage or treat. Unfortunately, however, biomarkers can be both an integral part of the pathologic process as well as an epiphenomenon. Hence, further research might be needed before they come into their own as indicators of disease outcome in humans. Nevertheless, they can be tracked when demonstrating proof of principle for a therapeutic intervention in pharmacodynamic models in animals as well as early studies in man. As surrogate markers, and until they are fully validated their use in drug development may be considered on a case-by-case basis. This presentation will provide some thoughts and insights into where and how the entire topic might fit in the regulatory context.

Agnes Klein
Manager, Clinical Evaluation Division, Biologics and Radiopharmaceuticals Evaluation Centre, Biologics and Genetic Therapies Directorate, Ottawa, Ontario, Canada

Dr. Klein received her medical degree from the University of Toronto. She trained in Endocrinology, Medical Biochemistry and Public/Community Health. Dr. Klein joined Health Canada and the Drugs Directorate in 1974 and has occupied many and varied scientific and management positions within Health Canada and its regulatory arms. Amongst these is having acted as the Director of the Bureau of Human Prescription Drugs and as Director of the Biologics and Genetic Therapies Evaluation Centre. Since April 2000, Dr. Klein has been with the Biologics and Genetic Therapies Directorate, initially as a Senior Medical Advisor and most recently as the Manager of the Clinical Evaluation Division, responsible for pre-market review as well as for decisions in respect of post-market events relating to biological/biotechnology agents. The Manager of the Clinical Division directs a large complement of clinical staff that is responsible for the evaluation of the clinical portion of the files submitted to Health Canada when manufacturers intend to market biological/biotechnology products. Dr. Klein is an active member of several medical and scientific organizations nationally and internationally. She is also a recent member of the DIA's Canadian Programme Steering Committee. Dr. Klein's special interests include the appropriate design of clinical trials and the ethical issues attendant to them. Dr. Klein is a member of Health Canada's Research Ethics Board.

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SESSION 4

Intersubject Variability In Drug Response

Intersubject Variability In Drug Action

Jacques Turgeon, Faculty of Pharmacy, University of Montreal, Montreal, Quebec Canada

Mr. Jacques Turgeon has been appointed Dean of the Faculté de Pharmacie, Université de Montréal on June 1st, 2000. He received his Bachelor degree in Pharmacy in 1983 from Laval University in Quebec City followed by a M.Sc. degree in pharmacokinetics and a Ph.D. degree in drug metabolism from the same institution in 1985 and 1988, respectively. He completed post-doctoral studies from 1988 to 1990 in the department of Clinical Pharmacology, Vanderbilt University in Nashville, USA, under the supervision of Dr. Dan M. Roden. He joined the Faculty of Pharmacy of Laval University in 1990 as an assistant professor. He was promoted to the rank of associate professor in 1993 and full professor in 1998. From March 1999 to May 2000, he was Senior Director of the Pharmacokinetics department at Phoenix International Life Sciences. Research interests of Dr. Turgeon have always been directed towards the study of factors responsible for intersubject variability in drug response. More specifically, he has developed expertise in the role of pharmacogenetics in cardiovascular drug actions. Among his favorite topics was the study of drug-drug interactions leading to pharmacodynamics modulation of antiarrhythmic drug action. Dr. Turgeon has integrated in his research approaches *in vitro* (patch-clamp technique, *in vitro* metabolism and molecular biology) models as well as designed and performed studies in healthy volunteers and patients. He has published more than 70 referred articles and more than 170 abstracts. Dr. Turgeon has received numerous prizes for his research activities as well as recognition by the students for his teaching skills. He is a member of numerous societies and has been acting on the committees of several granting agencies for several years. He has been the Director of the Quebec Cardiovascular Network of the FRSQ and the Research Director of the Quebec Heart Institute, Laval Hospital.

Jacques Turgeon
Professor and Dean, Faculty of Pharmacy, University of Montreal, Montreal, Quebec Canada

Several factors modulate the response obtained following administration of a particular drug to a particular patient at a particular time. This statement argues against the *one size fits all* concept and clearly defines the need for individualized drug therapy. We know, in everyday practice, that patients are treated in the setting of multiple drugs administered, concomitant diseases, and varying physiological and pathological conditions that all modulate drug effectiveness and toxicity. In addition, we have learned in the recent years that the disposition of drugs can differ from one individual to another due to polymorphisms in drug metabolism enzymes and transporters. One of the best examples is the cytochrome P450 superfamily. We currently believe and work at demonstrating that knowledge of patient's phenotype or genotype may help prevent drug side effects and even drug-drug interactions in specific subpopulations. During this conference, two examples of drug toxicity related to intersubject variability in drug action will be provided for CYP3A substrates and CYP2D6 substrates. In the first example, we will demonstrate how a change in total and intracellular concentrations of CYP3A substrates may alter high affinity binding to K^+ channel proteins in cardiac tissue thus explaining cardiac toxicity of non-antiarrhythmic agents. We will discuss involvement of P-glycoproteins and other transporters in specific tissue to better understand the underlying mechanism. In our second example, modulation of venlafaxine metabolism, a CYP2D6 substrate, will be used to demonstrate again intersubject predisposition to cardiac toxicity. This time, Na^+ channel block will be the target for drug toxicity. Stereoselective metabolism and formation of active metabolites will also be discussed. Finally, we will suggest strategies taking advantages of genetic polymorphism and modulation of cytochrome P450 activity to improve, in the near future, drug efficacy. With this new knowledge in hand, we shall be able to define better pharmacotherapy for our patients.

Pathophysiological Changes And Intersubject Variability

Fakhreddin Jamali, Professor and Associate Dean, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

It is an axiom of pharmacotherapy that there is a direct or indirect positive relationship between the dose and/or concentration and pharmacological response. Indeed, a typical clinical trial, which is carried out under controlled conditions is expected to yield such an outcome. Recent findings, however, suggest that under uncontrolled conditions, i.e., during the post-market period when real patients are treated, dose-response relationships may not always exist due, sometimes, to down-regulations (reduced response) of the involved receptors. This is attributed to the pathophysiological conditions and the presence of endogenous mediators that result in pharmacodynamic and/or pharmacokinetics outcome changes. For example, elderly patients seem to have reduced response to some cardiovascular therapies; control of blood pressure in patients with arthritis is problematic, and patients in acute pain have delayed response to oral analgesics. Mechanisms behind these findings are not quite clear. Nevertheless, all of these conditions coincide with altered production of endogenous mediators such as C-reactive protein, tumor necrosis factor and other cytokines as well as nitric oxide. Interestingly, very recently, mortality secondary to various cardiovascular diseases has been linked to these pro-inflammatory mediators. It is remained to be explored whether, or not, this increased mortality is due to a reduced effectiveness of the therapy. Nevertheless, any altered regulation of receptors is expected to result in unexpected changes in response to pharmacotherapy. This should contribute to increased inter-subject variability in response. Altered mediators concentrations is a common response to many mild to severe and diagnosed or undiagnosed conditions such as stress, infection, arthritis, asthma, aging and cardiovascular conditions. This may be a major source of inter-subject variability in response involved during both drug development and pharmacotherapy.

Fakhreddin Jamali
Professor and Associate Dean, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

Dr. Jamali (Doctor of Pharmacy, University of Tehran, Iran; MSc, pharmaceutics, PhD, pharmacokinetics, University of British Columbia, Vancouver, Canada) is a professor and the associate dean at the Faculty of Pharmacy and Pharm. Sci., University of Alberta. He joined the faculty at the University of Alberta in 1981. His research interests include effect of pathophysiological changes on the action and disposition of drugs, stereochemical aspects of drugs action and disposition, basic and clinical pharmacology of anti-rheumatic, analgesic and cardiovascular drugs, and toxicology of nonsteroidal anti-inflammatory drugs. He has published over 160-refereed articles and has been an invited speaker at many conferences, and has trained over 25 PhDs. He has served, as a Theme Leader in the Canadian Arthritis Network (Networks of Centres of Excellence), is a Fellow of American Assoc. Pharm. Sci. and American College of Clin. Pharmacol. and for his research achievements, he has received the McKeen Cattel Memorial Award of the American College of Clin. Pharmacol., the McCalla Professorship of the University of Alberta, the McNeil Award of Assoc Canadian Faculties of Pharm., DuPont Research Leadership Award of the Canadian Soc. Pharm. Sci.. Dr. Jamali has served as a consultant and/or a member of the board of directors of many pharmaceutical houses. He is 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.ualberta.ca/~csps), assoc editor of Eur. J. Med. Chem., and has served in the editorial board of J. Clin. Pharmacol. Chirality and Am. J. Therapeutics and AAPS PharmSci. He teaches pharmacokinetics and is involved in pharmacy curriculum development.

Natural Health Products and Drug Disposition

Brian C. Foster, Senior Science Advisor, Office of Science, Therapeutic Products Directorate, Health Canada, Ottawa, Ontario, Canada.

There are many classes of phytochemical xenobiotics with claimed health benefits. Traditional uses of most natural health products (NHPs) have proven safety, but their modern/current pattern of consumption and uses in the global context has changed. Many consumers generally regard NHPs as safe but anecdotal and published reports suggest that they can affect absorption, metabolism, distribution and elimination of drugs and other xenobiotics that enter the body. Problems can occur when drugs and other xenobiotics, which compete for the same active sites or shunt the products and their metabolites through other pathways, are taken together. The primary objective was to establish the potential of various NHPs to affect the metabolism of human cytochrome P450 isoforms *in vitro*, antibiotic resistance and gene expression to ascertain the risk potential of these products for generating possible adverse interactions with conventional drugs. Roughly 95% of the NHPs examined markedly inhibited 2C9, 2C19, 2D6, 3A4/5/7-mediated metabolite formation. Of the NHPs examined, some goldenseal and SJW, valerian root, garlic, and *Echinacea* products had high inhibitory activity. Many NHPs also strongly affected P-glycoprotein-mediated transport. Although some St. John's wort (SJW) and fresh garlic extracts stimulated the sensitivity of some antibiotics in the cultures tested, other antibiotics were less effective suggesting effects on bacterial drug transport. The relative concentrations of phytochemical markers in many of the products tested varied greatly emphasizing the need for quality assurance testing and broader testing of related products. High throughput methods can assess the probability of identifying interactions occurring between NHPs and conventional drugs metabolized by the same isozyme, but cell culture gene expression and functionality testing is required for complete characterization. The safety and efficacy of therapeutic products, when taken concomitantly with natural health products need to be examined further through extended 2-3 week clinical studies.

Brian Foster
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Dr. Foster is a Senior Science Advisor in the Office of Science, Therapeutic Products Directorate, Health Canada. He received his Ph.D. in Medicinal Chemistry at the University of Alberta in 1981 with Professor R.T. Coutts through research on alternative models for drug interactions and metabolism. Since joining Health Canada, his research has been in the areas of toxicology and drug disposition. His current research interest is in the area of drug disposition, pharmacogenetics, and how natural health products affect the safety and efficacy of conventional therapeutic products. Dr. Foster is an Adjunct Professor, Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa where he has a joint Health Canada - University of Ottawa laboratory. He has assembled a multi-disciplinary team with expertise in clinical care of elderly and terminally ill populations, ethnobiology, pharmacogenetics, pharmacokinetics, and physiology to study the effect of herbal and nutraceutical products on antibiotic resistance, and drug disposition in HIV/AIDS and elderly populations. He teaches drug disposition and pharmacogenetics. http://www.uottawa.ca/academic/med/cellmed/foster.html

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Multiple mechanisms by which grapefruit juice alters drug disposition and response in humans

David G. Bailey
Research Scientist, Department of Medicine, London Health Sciences Centre, London, Ontario, Ontario

Molecular mechanisms in both the small intestine and liver are capable of determining oral drug bioavailability. This may occur by presystemic conversion of drug to metabolite by cytochrome P4503A4 (CYP3A4) in enterocytes and hepatocytes. Grapefruit juice can augment oral drug bioavailability by reducing enterocyte CYP3A4 protein content without altering hepatocyte CYP3A4 activity. Since enterocyte CYP3A4 mRNA content was not changed, the interaction was caused by enhanced enzyme degradation, likely from mechanism-based enzyme inactivation. Since the return of CYP3A4 activity requires de novo protein synthesis, grapefruit juice produces prolonged duration of inhibition of CYP3A4 activity. Transporters may determine oral drug bioavailability. P-glycoprotein (P-gp) is an efflux pump located on the luminal surface of enterocytes and the bile canalicular membrane of the hepatocytes. P-gp pumps drugs back into the small intestine. Information from a number of sources supports an inhibitory effect by grapefruit juice on intestinal P-gp activity to increase oral drug bioavailability. Organic Anion Transporting Polypeptides (OATPs) are drug uptake transporters located on the luminal membrane of enterocytes and sinusoidal membrane of hepatocytes. OATPs enable drug uptake from the gastrointestinal tract into the portal circulation and facilitate the movement of drug from portal circulation into hepatocytes. Recently, grapefruit, orange and apple juices and certain constituents (furanocoumarins and bioflavonoids) were demonstrated to be potent inhibitors of OATPs. Clinically, these juices were shown to reduce the oral bioavailability of the non-metabolized antihistamine, fexofenadine, likely by inhibition of intestinal OATPs, which would represent a new mechanism of food-drug interactions.

David Bailey
Research Scientist, Department of Medicine, London Health Sciences Centre, London, Ontario, Ontario

Dr. Bailey completed his undergraduate and graduate education at the University of Toronto. He is currently Associate Professor of Medicine (Division of Clinical Pharmacology) and of Physiology & Pharmacology at the University of Western Ontario and Research Scientist, Lawson Health Research Institute at the London Health Sciences Centre. For the past 15 years, Dr. Bailey's major focus of research has been the investigation of mechanisms responsible for drug interactions in humans. One of his more noteworthy findings was that grapefruit juice could increase the oral bioavailability and potential for adverse effects of medications because of inhibition of an important enzyme of drug metabolism located in the gastrointestinal tract (CYP3A4). During his undergraduate years at the University of Toronto, Dr. Bailey was a dedicated athlete. He had the honour to represent Canada on several national track and field teams. He was the first Canadian to run the mile in less than four minutes.

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SESSION 5

Novel Excipients And Formulation Technologies

Jasmine Prucha
Sales, Pharmaceutical Division, Gattefossé Canada Inc., Baie-d'Urfé, Québec, Canada

Jasmine Prucha is Director of Sales Pharmaceutical Division at Gattefossé Canada Inc. a subsidiary of Gattefossé s.a. and a corporate supporter of CSPS. She joined the company in June 1997 and was later named as national Sales Director Cosmetic and Pharmaceutical ingredients (2000-2003). Jasmine received her M.Sc. in Food Science and Agricultural Chemistry in 1986 from McGill University. Her graduate work under the direction of Dr. Inteaz Alli focused on protein chemistry leading to publication of scientific articles related to isolation and characterization of vegetable proteins. After graduation, she spent three more years at McGill as research assistant in Animal Science and Food Science Departments. Her sales career began in 1989 as Technical Sales Representative, selling scientific instruments such as X-Ray Diffraction, Thermal Analysis, HPLC, and Atomic Absorption intended for research laboratories. Jasmine has since held sales positions in other disciplines including specialty chemicals, nutritional ingredients, cosmetic actives and pharmaceutical excipients. She draws from past experiences to bridge knowledge from multiple disciplines and enjoys working with formulation scientists in development of oral, topical and suppository dosage forms.

Amyn Sayani
Team Leader, Pharmaceutical Development, GlaxoSmithKline, Inc., Mississauga, Ontario, 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 primarily oral liquids, intranasal and topicals products. The team is involved in the development of new chemicals and product line extensions, from preclinical stages to launch. Prior to joining GSK Canada, Dr. Sayani served as a Research Investigator at Therics, Inc., in Princeton, NJ, evaluating the feasibility of a novel three-dimensional fabrication technology for programmed release of therapeutic peptides and proteins. Dr. Sayani received his BSc in Pharmacy in May 1992 and his Ph.D. from Rutgers University, New Jersey, in January 1998. Dr. Sayani has 15 publications in journals and conference proceedings, and four patent applications pending. Dr. Sayani is a member-at-large of the Executive of the Canadian Society for Pharmaceutical Sciences, and a guest lecturer in the Faculty of Pharmaceutical Sciences at the University of Toronto.

Lipid-Based Vehicles for Oral Delivery

Duncan Q.M. Craig, Professor and Chair in Biophysical Pharmacy, The School of Pharmacy, The Queen's University, Medical Biology Centre, Belfast, Northern Ireland, United Kingdom

The formulation of drugs into lipid-based dosage forms for oral administration presents a number of opportunities for both slow and rapid release. In this presentation, the incorporation of drugs into two such lipids, Gelucire 44/14 and Gelucire 50/13 will be highlighted. The Gelucires comprise a mixture of glycerides with mono- and diesters of fatty acids and polyethyelene glycol (PEG), with the two-descriptor numbers relating to the approximate melting point and HLB value respectively. Despite the compositional similarity of these two materials, their drug release behaviour is very different, with Gelucire 50/13 being associated with slow release while Gelucire 44/14 has been shown to increase bioavailability of poorly soluble drugs. The presentation will outline the solid-state characterisation of Gelucire 50/13 using thermal and microscopic methods, with particular emphasis on the hitherto poorly explored issue of how drugs influence the structure of the lipid and *vice versa*. More specifically the effect of incorporating the model drugs paracetamol and caffeine will be discussed, showing that despite the similar hydrophilicity of these two drugs their effects on the lipid and indeed their own physical form within the matrix show very significant differences. The dissolution behaviour will then be described; with emphasis on deconvoluting, the mechanisms involved using both modelling techniques and direct measurement of erosion, water uptake and dimensional change. The use of Gelucire 44/14 will then be described in the context of formulating a liquid drug, Vitamin E. The use of low temperature modulated temperature DSC as a means of characterising this material will be outlined in conjunction with differential interference contrast and confocal microscopy. The results of a human bioavailability study comparing the Gelucire 44/14 formulation to a standard soft gelatin capsule will be outlined.

Duncan Craig
Professor and Chair, Biophysical Pharmacy, The School of Pharmacy, The Queen's University, Medical Biology Centre, Belfast, Northern Ireland, United Kingdom

University of Bath, 1981-1984 Bachelor of Pharmacy (Hons); First Class, Registered pharmacist since 1985; Postgraduate Studies at School of Pharmacy, University of London, 1985-1988; 1988 - 1991: Teaching and Research Assistant, School of Pharmacy, University of London; 1991 - 1996: Lecturer, School of Pharmacy, University of London; 1996 - 1998: Senior Lecturer, School of Pharmacy, University of London; 1998 - 1999: Reader in Pharmaceutical Materials Science, School of Pharmacy, University of London; 1999 - : Chair in Biophysical Pharmacy, Head of Pharmaceutical Materials Science Group, The School of Pharmacy, The Queen's University of Belfast. Research interests: Physical characterisation of drugs, dosage forms and biological materials in relation to performance, with particular emphasis on the development and use of novel thermoanalytical techniques. Specific areas of interest include development of novel techniques for dosage form characterisation (e.g. T_{zero} DSC, microthermal analysis), development of drug delivery systems using for example lipids and hydrophilic polymers, protein formulation and delivery especially in terms of freeze drying and biophysical analysis including studies on cystic fibrosis mucus and erythrocyte preservation. 104 full research papers, 189 conference abstracts, 2 books, 5 book chapters, 12 articles/editorials, approximately £3m in research grants (circa 50% from peer reviewed bodies), 32 invited conference presentations plus numerous invitations to speak at academic institutions Editor of Thermochimica Acta and Journal of Pharmacy and Pharmacology, member of editorial board of International Journal of Pharmaceutics and Journal of Pharmaceutical Sciences. Former winner of BPC Science award. Member of Unilever Expert Advisory Panel. Member of Queen's University Research Committee overseeing research/RAE strategy for university.

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Formulation Development of New Chemical Entities using Special Excipients and Technologie

Kwok Chow, Director, Formulation Development, Patheon Inc., Mississauga, Ontario, Canada

Many new chemical entities (NCEs) exhibit challenging solubility/bioavailability, stability or physical attributes (e.g. large particle size, polymorphism, poor compression). The use of non-traditional technologies and ingredients can play a significant role in achieving speed, budget and clinical goals in developing these NCEs. For example, solublization or suspending vehicles for reconstitution are useful for powder in a bottle (PIB) applications to support first time in man studies. These techniques can extend the use of PIB from high solubility to low solubility or moisture sensitive compounds. For poorly bioavailable NCEs, simple solutions such as conventional size reduction techniques, strategic choice of salts/polymorphs/solvates, and effective use of excipients and optimization of processes can allow rapid, low cost development. Less conventional technologies including particle/crystal engineering, complexation, solid dispersion, novel particle size reduction techniques, lipid-based technologies, and alternate delivery systems (e.g. nasal spray with/without enhancers) are highly effective in delivering the drug *in*-vivo. Strategies can be employed to stabilize or protect temperature and/or moisture sensitive compounds to avoid costly packaging development / manufacturing and achieve the intended product shelf-life and storage conditions. Typically, special excipients to create a 'micro-environment' to stabilize NCEs can be employed and/or moisture protection film coating can be applied (at ambient or slightly elevated temperatures). Successful development of NCE formulations is a balance between time, cost, and clinical goals. Close collaboration between formulation and chemical development teams and appropriate use of preformulation results can impact the timeline and outcome of the development program. Simple approaches are recommended for less challenging molecules. Timely, strategic decisions are often required to employ more complex development programs, based on data from conventional or special techniques (e.g. x-ray diffraction of drug substances and products). Scientific knowledge / experience and efficient experimental design (e.g. statistical design of experiment) are key success factors for bringing molecules to market.

Kwok Chow
Director, Formulation Development, Patheon Inc., Mississauga, Ontario, Canada

Dr Chow is the Director of Formulation Development at Patheon Inc, Canada. He received his BS in Pharmacy from the University of Minnesota, USA, and his MSc and PhD in Industrial Pharmacy from the University of Toronto, Canada. Dr. Chow began his formulation development career at Glaxo in 1988. During his 13 years of service, he held various positions with increasing responsibilities and was leading the development of conventional and novel dosage forms (including tablets, capsules, liquids, suspensions, nasal sprays, powder for reconstitution and fast dissolving formulations). Dr. Chow has successfully introduced new chemical entities and line extension products in the U.S., Canada, Europe, Japan and other Asian Pacific countries. Dr Chow was an international CMC project leader for the development of new chemical entities. He was responsible for formulation and process development of new compounds, and coordinating activities with Chemical Development and CMC Regulatory Affairs. Dr. Chow had successfully planned and prepared CMC technical dossiers for clinical studies or regulatory approvals. In his current role as Director of Formulation Development at Patheon, Dr. Chow oversees the formulation development activities at the Toronto Region Operations. He also manages a dynamic team of Managers, Scientists and Technologists to provide quality services for pre-formulation, formulation, clinical/registration batch manufacture, throughout the drug development process. Dr. Chow teaches and coordinates both undergraduate and graduate courses in formulation development at the University of Toronto. He also trained industrial graduate students. He is the author or co-author of a number of patents, research articles and abstracts.

Use of Nanoparticles as an Enabling Technology in Dosage Form Design

Eugene R. Cooper, Executive Vice President and Chief Technical Officer, Research & Development, Elan Drug Delivery Inc., King of Prussia, Pennsylvania, USA

The current trend in the properties of drug candidates coming out of drug discovery is that a significant fraction of these molecules is poorly soluble in water. In fact, the percentage is approaching fifty percent in some companies. Traditional approaches to dealing with this delivery problem are to micronize the drug substance, find a solvent system to solubilize the drug, or just discard the drug candidate. If the drug crystals are reduced in size to the submicron domain and properly stabilized, many of the delivery problems associated with poor aqueous solubility can be overcome. The benefits of such an approach will be illustrated along with the technology used to make pharmaceutically acceptable dosage forms.

Eugene Cooper

Executive Vice President and Chief Technical Officer, Research & Development, Elan Drug Delivery Inc., King of Prussia, Pennsylvania, USA

Dr. Eugene Cooper is Executive Vice President and Chief Technical Officer of Research and Development for Elan Drug Delivery, Inc. He joined Elan in 1998 through the acquisition of NanoSystems where he was a founder and served as Chief Technical Officer and Vice President, Research & Development. He was previously Executive Director of Pharmaceutical Sciences worldwide at Sterling Winthrop Pharmaceutical Research Division (1989-1994), where he developed nanoparticle technology to provide Sterling Winthrop a competitive edge in research and development. Dr. Cooper has also worked in drug delivery in the field of ophthalmology at Alcon Labs and cutaneous transport at Procter and Gamble and as one of the founders of Theratech. Dr. Cooper received a National Science Foundation Postdoctoral Fellowship in Chemistry after receiving a Ph.D. in Theoretical Chemistry from Iowa State University. His undergraduate training was in mathematics and physics at Austin College. He is the author of numerous research articles and patents covering a wide range of areas from nonequilibrium statistical mechanical to x-ray diagnostic imaging.

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Overview Of Novel Oral Drug Delivery Systems: Rapid Dissolve And Controlled Release

Sophie-Dorothée Clas, Senior Investigator, Pharmaceutical Research & Development, Merck Frosst Canada & Co., Kirkland, Quebec, Canada

The goal of the presentation is to provide a general overview of the different technologies available for fast dissolve and controlled release formulations. Fast dissolve dosage forms are drug delivery systems that dissolve or disintegrate in the buccal cavity in less than one minute, without the need for water. They are different from chewable tablets in that the fast-dissolve or melt-in-your mouth formulations do not require significant mechanical force to disintegrate. They are generally very friable relative to conventional oral tablets and often require special packaging to protect from breakage. In addition, as they are designed to disintegrate on contact with the saliva in the mouth, they can require moisture-barrier packaging to protect them from moisture. Controlled oral delivery systems are drug delivery systems that modulate the rate of release of the drug. These include sustained release, where the drug is released at a specified rate over a defined period; modified release, where a portion of the drug is released immediately while the remainder is released as sustained release; delayed release, where drug is released after a predetermined delay; and pulsatile release formulations, where the drug is released in small amounts.

Sophie-Dorothée Clas
Senior Investigator, Pharmaceutical Research & Development, Merck Frosst Canada & Co., Kirkland, Quebec, Canada

Dr. Clas joined the Merck Frosst Canada & Co., Centre for Therapeutic Research, in 1991 and is presently Senior Investigator, in the Pharmaceutical Research & Development Department. She leads the materials characterization/ preformulation group in PR&D and is responsible for the physico-chemical characterization of all new drug development candidates at Merck Frosst. The group uses many different techniques to characterize both the drug substance and product: including thermal analysis (differential scanning calorimetry, thermogravimetry, thermomechanical and dynamic mechanical analysis), temperature and humidity X-ray powder diffractometry, spectroscopic analysis (FT-IR/Raman, UV, SSNMR) and HPLC. Dr. Clas is also adjunct professor in the Faculty of Pharmacy at the University of Montreal giving lectures on materials characterization and thermal-analytical methods. She participates in the Concordia University COOP program, supervising the student work terms. Her research interests include among others, the thermodynamic stability of polymorphs, detection of the physical form of the drug in solid oral dosage forms, and materials characterization of polymers, specifically dynamic mechanical properties of polymers such as excipients, coatings and gelatin. Dr. Clas obtained a PhD under the direction of Dr. A. Eisenberg in Polymer Chemistry (1985) for the study of the structure-property relationships of styrene-alkoxide ionomers using dynamic mechanical spectroscopy. After spending a year on a NSERC postdoctoral fellowship at Queen's University in Kingston with Dr. K.E. Russell studying the effect of branch length and concentration on the crystallization and mechanical properties of ethylene copolymers, Dr. Clas returned to McGill University in 1986 as a Research Associate. From 1986 to 1990, Dr. Clas worked in collaboration with Dr. L.E. St-Pierre and Dr. G.R. Brown on the study of bile acid sequestering resins for cholesterol-lowering applications. From January 1990 until she joined Merck Frosst, Dr. Clas worked as a Research Associate at the Pulp and Paper Research Institute of Canada. She joined Merck Frosst Canada & Co. in 1991 as Senior Research Chemist.

Use of permeation enhancer excipients in oral formulations: A case study with antisense oligonucleotides

Lloyd G. Tillman, Director, Pharmaceutical Development, Isis Pharmaceuticals, Inc., Carlsbad, California, USA

The oral absorption of hydrophilic macromolecules such as peptides, proteins, heparins and oligonucleotides is limited due to poor permeability across the GI membranes. Generally the bioavailability of these macromolecules is nil without the use of adjuvant permeation enhancers. Most permeation enhancers enable the paracellular path for absorption across mucosa by means of opening of the epithelial tight-junctions. One such enhancer, the fatty acid sodium caprate, has been extensively studied for this application and was found suitable for oral delivery of oligonucleotides. The development of this enhancer based oral dosage form required a variety of studies across animal models to ascertain those formulation requirements unique for sodium caprate mediated absorption of oligonucleotides. Starting with rat models, then to monkeys, dogs and ultimately human studies, both the dosing requirements (i.e., drug vs. enhancer) and the synchronized release pattern of these components were determined. These studies initially use solution presentations of the formulation wherein the timing for the enhancer release – relative to the drug – is directly controlled. The solution data underscore the performance criteria or specifications necessary for a controlled release solid dosage form. This CR solid dosage form development initially targets *in vitro* specifications that are later qualified by *in vivo* testing in man. This qualification is accomplished by tracking the dosage units, using scintigraphy, to address the issue of gastric emptying and the time / location of dosage unit disintegration in the intestine. The scintigraphy is accomplished by means of incorporating samarium oxide tracer into the formulation matrix, which is then irradiated to create a gamma-emitting isotope. The scintigraphy data allows for the deconvolution of the plasma pharmacokinetic data giving assurance on this case study's calculated oligonucleotide oral bioavailability to be 7%.

Lloyd Tillman
Director, Pharmaceutical Development, Isis Pharmaceuticals, Inc., Carlsbad, California, USA

Dr. Lloyd Tillman is Director of Pharmaceutical Development at Isis Pharmaceuticals, Inc., Carlsbad, CA, where he is responsible for the formulation research and development of oligonucleotide drug products. He manages CMC activities relating to drug development, clinical supply and registration. Prior to joining Isis, Dr. Tillman worked at the US Food and Drug Administration from 1994 to 1997 as the Associate Director overseeing the Division of Product Quality Research within CDER. His group focused to derive data in support of SUPAC guidelines and the biopharmaceutics drug classification system initiative. Dr. Tillman was previously a Group Leader over solid dosage formulations at Burroughs Wellcome Co., Greenville, NC (1987-1994). Dr. Tillman received a Ph.D. in Pharmaceutics from the University of Georgia in 1987.

POSTER PRESENTATIONS

The Science Of Drug Discovery & Development

CSPS Posters will be on display in the Foyer and Regence BC from 8-5 Thursday.
AFPC Posters will be on display in the Foyer and Regence BC from 8-5 Friday.

Presenters will be available by their posters during the coffee and lunch breaks.

1. PERCUTANEOUS PENETRATION OF SUNSCREEN BENZOPHENONE-3 AND MOSQUITO REPELLENT N,N-DIETHYL-M-TOLUAMIDE (DEET) IN VITRO

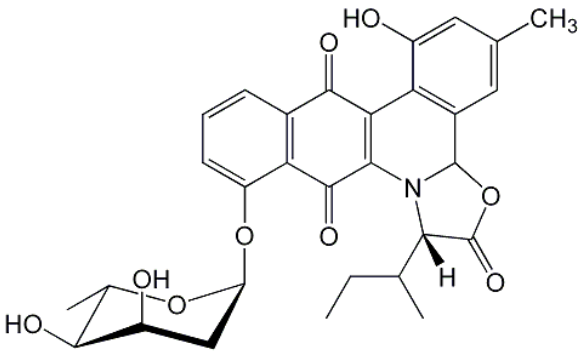
***Daryl Fediuk*, Xiaochen Gu, Faculty of Pharmacy, University of Manitoba, Winnipeg, Canada**

Purpose: Benzophenone-3 and DEET are essential components in commercial sunscreen and mosquito repellent preparations respectively. Their characteristics in percutaneous absorption are virtually unknown. We carried out a series of in vitro studies to evaluate the percutaneous penetration and interaction of these two compounds at different temperatures. **Methods:** In vitro penetration studies were performed in Franz-style diffusion cells at 25, 37, and 45 °C, using pH 7.4 phosphate buffer and synthetic poly(dimethyl siloxane) (PDMS) membranes. Benzophenone-3 and DEET in propylene glycol at 1, 2.5, 5, 10 mg/mL were applied separately or in combination to donor cells. Samples were collected hourly from receptor cells for 6 hours. Concentrations of benzophenone-3 and DEET were analyzed simultaneously using HPLC. **Results:** Both benzophenone-3 and DEET penetrated across the PDMS membranes in all diffusion studies. The total penetration amount for both compounds correlated linearly to the concentrations tested (r^3 0.95). As a single entity, the penetration at varying concentrations and temperatures was 3-10 % for benzophenone-3 and 2-4 % for DEET respectively. Using the combination, the penetration at varying concentrations and temperatures was 4-10 % for benzophenone-3 and 2-5 % for DEET respectively. The presence of both compounds in the same preparation resulted in synergistic penetration of benzophenone-3 and DEET, indicating interaction of the compounds in percutaneous absorption. Higher temperatures also facilitated the diffusion process and hence enhanced percutaneous penetration of both components. The greatest increment of penetration was 10 % for benzophenone-3 at 5 mg/mL and 45 °C. **Conclusion:** The penetration of benzophenone-3 and DEET across the PDMS membranes was enhanced when they were present simultaneously. The potential percutaneous interaction and synergistic penetration of sunscreen chemicals and mosquito repellent DEET from either concurrent application or composite products thus requires further systematic evaluation.

2. PREPARATION AND EVALUATION OF NOVEL ANTIBIOTICS PRODUCED BY *S. VENEZUELAE*

***René Douce*t and David L. Jakeman, College of Pharmacy, Dalhousie University, Halifax, Canada**

Purpose: To investigate the structure-activity relationship of a novel series of polyketide derived antibiotics isolated from *Streptomyces venezuelae.* The compounds were structurally related to jadomycin B and tested for anti-microbial and anti-cancer activity. **Methods**: A spore suspension of *Streptomyces venezuelae* ISP5230 DWB was dispensed into MYM media and grown for 20-24 h at 30° C with shaking. An aliquot of the resulting vegetative inocula was added to defined media containing D-galactose, an L-amino acid, and mineral salts. The bacteria were grown using isoleucine, glycine, 2-aminoisobutryic acid, b-alanine, alanine, tryptophan, threonine, cysteine, and phenylalanine as sole nitrogen sources. Ethanol shock induced production of the antibiotic.: The bacterial cell paste was filtered and the antibiotics were extracted from the aqueous phase by ethyl acetate extraction. The compounds were further purified by flash column chromatography with a step-wise gradient of dichloromethane-methanol. Analytical HPLC was used to assess purity. Evaluation of the compounds for anti-microbial activity was performed on bacterial lawns of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus fecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli* strains. **Results**: Jadomycin and jadomycin B (50 mgL^{-1}) were obtained from the isoleucine-containing media. A multitude of compounds were observed with other amino acids, several of these were isolated, and significant antimicrobial activity was observed with analogues extracted from the media containing tryptophan and threonine. Based on the recent biosynthetic pathway described for jadomycin B biosynthesis we have proposed structures of the related tryptophan and threonine analogues. Anti-cancer assays on jadomycin B samples show significant activity. **Conclusion**: We have isolated a number of structurally related, novel, carbohydrate-containing, polyketide derived compounds from *Streptomyces venezuelze*. Several of the compounds show antimicrobial and anti-cancer activities. **Acknowledgement**: Financial support for this project was provided by the Merck Company Foundation National Summer Student Research Program.



Jadomycin B

3. SYNTHESIS AND BIOLOGICAL ACTIVITY OF SEMICARBAZONE DERIVATIVES: ACTIVE VERSUS NON-ACTIVE ANTICONVULSANTS

***S. Pandeya*[1], Sonia Kohli1 and James P. Stables[2], 1. Emeritus Fellow, AICTE and Professor of Pharmachemistry, Institute of Technology, Banaras Hindu University, Varanasi-221005. 2. Preclinical Pharmacology Section, Epilepsy Branch NIH, Bethesda, Maryland, USA**

Purpose: To synthesize novel class of anticonvulsant drugs. In recent years, semicarbazones are making headway in the field of anticonvulsants. In the laboratories of Pandeya and Dimmock, many such derivatives are continuously being synthesized. **Methods**: The strategy of the synthesis has been to design molecules that possess (i) a hydrophobic aryl center (ii) a hydrogen bonding domain (iii) a two-electron donor system and (iv) a distal hydrophobic center, which may be or may not be essential for bioactivity. In order to ascertain which one is important for this activity, several modifications, at all the points in the molecule are being undertaken. Aryl rings containing derivatives have been synthesized. Some isatin Schiff bases and N-ethyl substituted aryl semicarbazones have been prepared. In the aryl semicarbazones, a chloro hydrophobic group is essential, blocking of -NH- with N-ethyl groups abolishes activity. This proves the importance of hydrogen bonding. A two-electron donor system is essential and present in all the compounds. The distal hydrophobic group influences the type of anticonvulsant activity, MES Vs scMET. This study has shown a new light in the concept of anticonvulsant activity of semicarbazones. These compounds also exhibit no sedative hypnotic activity. **Result**: The lead compound identified is p-chlorophenyl substituted semicarbazone. **Conclusion**: The newly identified p-chlorophenyl substituted semicarbazone will provide further beneficial modification in the design of semicarbazones as anticonvulsants.

4. DEVELOPMENT AND PHYSICO-CHEMICAL CHARACTERIZATION OF A POLYMER-LIPID HYDROGEL IMPLANT FOR DRUG DELIVERY

***J Grant*[1], C Allen[1,2] (Department of Pharmaceutical Sciences[1], Department of Chemical Engineering and Applied Chemistry[2], University of Toronto)**

Purpose: The goal of our research is to develop an implant system from biocompatible biodegradable materials that provides prolonged and controlled release of hydrophobic drugs. In order to accomplish this, a two-tiered system has been designed wherein the drug is concentrated in poly (*d, l*-lactide) nanoparticles localized within a chitosan-lipid matrix. The nanoparticles act as "cargo space" for drug while the matrix provides a barrier to ensure controlled release and prevent dose dumping. **Methods:** Formulation, swelling, pH and stability studies were performed on the implant system. The morphology of the implant system was studied by SEM. The distribution of lipid in the polymer matrix was analyzed by TOF-SIMS. The model hydrophobic anti-cancer agent, paclitaxel, was incorporated and the release kinetics was studied in biologically relevant media. **Results:** We prepared a physically crosslinked hydrogel from a chitosan-phospholid blend. Our studies revealed that the ratio of lipid: chitosan as well as the nature of the lipids employed (e.g. PS versus PC) control the physico-chemical properties (e.g. stability, swelling) of the implant. Specifically, the swelling ratios varied from 6.78-104.52 depending on polymer-lipid compositions. The effect of additives such as PEG and Dextran were also studied. The ideal formulation was found to be 1:0.83 (w:w) chitosan:phosphatidylcholine. SEM demonstrated changes in surface and cross-sectional morphology of the implant following incubation in serum. TOF-SIMS analysis revealed a homogeneous lipid distribution throughout the polymer matrix. The total drug released from the implant system in PBS (with 2 mg/mL lysozyme) was 30 % over a one-month period. **Conclusion:** An effective implant system has been designed with slow and controlled release of Paclitaxel over a one-month period. Future studies will include the study of the molecular interactions (hydrogen bonding, hydrophobic and ionic) that stabilize the system as well as further study of drug loading and release.

5. USE OF PHYSICO-CHEMICAL METHODS TO DESIGN A POLYMER- BASED FORMULATION FOR THE ANTICANCER DRUG ELLIPTICINE

***J. Liu*[1], Y. Xiao[1], C. Allen[1,2,] 1. Department of Pharmaceutical Sciences; 2. Department of Chemical Engineering and Applied Chemistry, University of Toronto**

Purpose: Ellipticine is a highly potent broad-spectrum anticancer agent. Clinical studies of this drug have been limited due to formulation difficulties mostly arising from low water solubility (0.153 mg/L). Recently, our laboratory has tried to establish a method of predicting polymer-drug compatibility as a means to guide formulation development. This method includes analysis by several physico-chemical techniques (X-ray, FTIR, DSC) and comparison of total/partial solubility parameters of drug and polymer. Polymers that were found to be compatible/incompatible with Ellipticine were used to formulate the drug. The drug formulations were studied in terms of stability, loading efficiency/capacity, and release kinetics. The results from formulation studies were then compared to findings from physico-chemical studies to determine if these methods accurately predict polymer-drug compatibility. **Methods:** Solubility parameters were calculated using group contribution methods. Polymer-Ellipticine blends were studied by FTIR, X-ray and DSC. Drug formulation was studied by light scattering and fluorescence assay for Ellipticine. **Results:**

Polycaprolactone (PCL) and Poly (DL-lactide) (PLA) were chosen as the hydrophobic blocks of the copolymers (PEO-b-hydrophobic block; 5000-b-4000). The physico-chemical methods and comparison of solubility parameters revealed that PCL is far more compatible with Ellipticine than PLA. The formulation characteristics agreed with the predicted results: loading efficiency for PCL micelles was 75.8% while that for PLA micelles was 1.9%. In-vitro drug release studies found the formulation based on PCL provided sustained release of 85 % of drug over 14 days. The PCL formulation increased the water solubility of Ellipticine more than six thousand fold. Conclusions: The combination of several physico-chemical methods and comparison of solubility parameters has been shown to be a useful tool for predicting compatibility. The PCL micelle formulation is a promising delivery system for Ellipticine. We now plan to synthesize novel copolymers that were predicted to be more compatible with Ellipticine based on comparison of polymer-drug solubility parameters.

6. RELATIVE LIPOPHILICITY OF SELECTED 1,2,4-THIADIAZOLE DERIVATIVES DETERMINED BY RP-HPLC AND QMPRPLUS™ SOFTWARE

***Jasmina Novakovic*[1], Ning Yuan Li[2], Jolanta Wodzinska[2], Tim Tam[2], Regis Leung-Toung[2], Michael Spino [1,2], Jake Thiessen[1], [1]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada, [2]Apotex Inc., Toronto, Ontario, Canada**

PURPOSE: In a search for improved, orally administered Factor XIIIa inhibitors, a series of 1, 2, 4-thiadiazole derivatives (N>20) are being tested. To predict their oral absorptivity, lipophilicity was estimated by three approaches, namely, reverse-phase high-performance liquid chromatography (RP-HPLC), a computer forecasting program (QMPRPlus™, Simulations Plus, Lancaster, CA, USA) and a traditional shake flask method. **METHODS**: Lipophilicity (log P) was estimated using RP-HPLC by correlation with the capacity factor (log k'). Thereby compounds were chromatographed on a C18 column using various isocratic mobile phase compositions (water and 25%-70% organic modifier (acetonitrile and methanol)). A linear relationship was established between log k' and the percentage organic modifier. Log k' values, extrapolated to 0% organic modifier (log k_w), were then correlated to log P obtained experimentally by the shake flask method (octanol/water; N=6), or to log P predicted by the commercially available software, QMPRPlus™. The latter employed lipophilicity estimation by the methods of Moriguchi (M log P) and a permeability model (S+ log P_{eff}). **RESULTS**: The 1, 2, 4-thiadiazole derivatives exhibited an experimental (shake flask) log P range from 1.5 to 4.5. The RP-HPLC log k_w values ranged from 0.9 to 4.3. The computer derived M log P and S+ log P_{eff} ranged from –1.4 to 2.5 and 1.3 to 4.9, respectively. The relationship between log k_w and log P obtained experimentally or predicted (M log P or S+ log P_{eff}) could be linearized by the equation: log k_w = a* log P + b. Correlations between the HPLC estimated lipophilicity (extrapolated log k_w) and either the experimental or computer predicted log P were very strong ($r^2 \geq 0.9$; $p<0.05$). **CONCLUSION**: RP-HPLC is a useful tool for identifying and selecting 1, 2, 4-thiadiazole derivatives with desirable lipophilicity. Log P values predicted for the series of compounds using QMPRPlus™ software also correlate very well with their experimental values.

7. MASS-BASED FRACTION COLLECTION OF SYNTHETIC PEPTIDES

***Linda Côté, Agilent Technologies (Canada),* Ralf Moritz, Agilent Technologies (Germany) ; Nicola O'Reilly, Cancer Research UK, London, United Kingdom**

Purpose: Synthetic peptides become more and more important as drug candidates in the treatment of a variety of diseases. In order to keep pace with the growing number of newly synthesized peptides, peptide purification should not represent the bottleneck in the drug discovery process. **Methods**: Mass-based fraction collection is an efficient technique for the purification of compounds with well-known masses. In contrast to fraction triggering with less specific detectors, in each run only the compound of interest is purified. Hence, it is not necessary to pick out target compounds out of a series of redundant fractions that have been collected. **Results**: Here we demonstrate mass-based purification of a series of synthetic peptides with the Agilent 1100 Series Purification System. The modular set-up of the system, a reliable fully automated delay volume calibration and a comprehensive software package assemble a versatile platform for purification tasks in a flow range up to 100 mL/min. We could successfully purify crude peptides by reverse phase HPLC from less than 1 kDa to more than 10 kDa, covering a pIrange from 4 to 13. **Conclusions**:

- Mass based fraction collection is a highly efficient technique for high-throughput purification of crude synthetic peptides.
- Due to an accurate delay volume calibration fraction collection with the Agilent 1100, Series Purification System is highly reliable.
- The presented system can cope with all kinds of peptides ranging from small to large, from hydrophilic to hydrophobic, from acidic to basic.
- Fraction collection triggered by predefined masses is advantageous over conventional less specific detectors since only the compounds of interest are being collected in each run. No additional time needs to be spending to pick out target compounds out of a series of redundant fractions. Furthermore, no fraction collector resources are wasted.

· No additional preparation steps are needed to obtain mass spectra within chromatographic runs. Peptide characterization with an ESI-MSD therefore is an alternative to MALDI-TOF.

8. INFLUENCE OF THE DELAY VOLUME ON FRACTION COLLECTION

***Linda Côté, Agilent Technologies (Canada)*, Udo Huber, Agilent Technologies (Germany)**

Purpose: For highest sample recovery in preparative HPLC, it is essential to determine the delay volume – or delay time – between the fraction collector and the detector used for fraction triggering. Usually the measurement of the delay volume is a tedious and error-prone process involving a highly concentrated dye and a stopwatch. **Methods and results**: We show a very easy, fast and automated way to perform a reliable delay volume calibration to optimize sample recovery in preparative HPLC. We further show why the delay volume control in a preparative HPLC instrument is crucial to achieve high recovery and avoid re-mixing of separated compounds. **Conclusions**:

· Fraction delay sensor gives possibility to perform delay volume calibration fast and automated.

· Especially important for mass-based fraction collection system due to influence of the make-up flow on delay time.

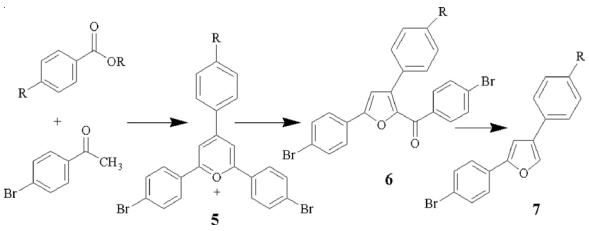
· Delay volume control is important to maximize recovery.

· Delay volume control is important to avoid re-mixing of separated peaks.

9. IMPROVED SYNTHESIS OF PYRYLIUM SALTS *EN ROUTE* TO MEDICINALLY IMPORTANT DIARYLFURANS

***Angélica Bello-Ramírez* and Lakshmi P. Kotra, Faculty of Pharmacy, University of Toronto**

Purpose: Substituted furans exhibit interesting biological and pharmacological properties. Alkyl and aryl furans have shown antibacterial, fungicidal, muscle relaxant, insecticide and enzymatic inhibition activities as well as nucleic acid binding properties. Routes to synthesize substituted diarylfurans, however, are limited. In this study, we synthesized substituted 2-aroyl-3, 5-diarylfurans and 2, 4-diarylfurans via pyrylium salts, starting from either substituted benzoic acids or substituted benzoic methyl esters instead aldehydes. We also propose a possible mechanism involved for the formation of these pyrylium salts. **Methods**: Pyrylium salts 5 were synthesized from substituted benzoic acids or substituted methyl benzoates and 4'-bromoacetophenone mediated by the Lewis acid catalyst ($BF_3{\cdot}OEt_2$) in toluene. The pyrylium salts were converted into 2-aroyl-3, 5-diarylfuran 6 with I_2 in basic media. In order to obtain the desired diarylfurans 7 from the compounds 6, we employed known Hallar-Bauer reaction. **Results**: Substituted pyrylium salts were spectroscopically identical whether the starting material was substituted benzaldehyde, substituted benzoic acid or substituted methyl benzoate. The yields of these compounds were higher if the starting material was the substituted methyl ester. The possible mechanism of formation of pyrylium salts 5 from esters or carboxylic acids involves several steps: the keto-enol equilibrium of acetophenone induced by the Lewis acid, the nucleophilic attack onto the carboxylic functionality, dehydrations and, as the last step, the internal cyclization. **Conclusions**: We synthesized pyrylium salts from aromatic substituted carboxylic acids and esters, and proposed the possible mechanism involved in their formation. These findings are useful for the synthesis of biologically relevant diaryl furans with different substituents (-X, -CN, -OMe, $-NO_2$), and opens doors to synthesize these molecules from readily available and easily manageable aryl carboxylates and/or their esters.

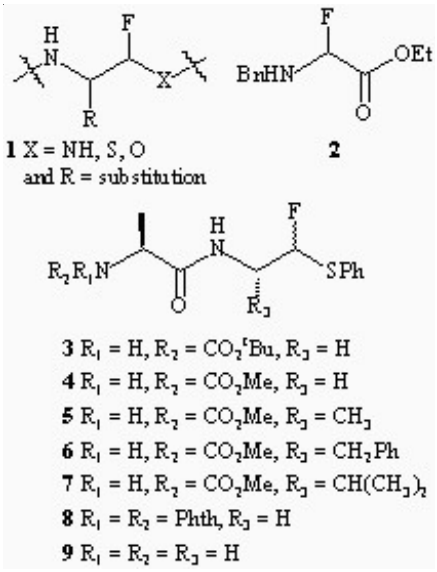


10. FLUOROPEPTIDOMIMETICS AS NOVEL MECHANISM-BASED PROTEASE INHIBITORS

***Subhash Annedi*[a], Sheeba Samson[a] and Lakshmi P. Kotra[a, b; a]Faculty of Pharmacy, Molecular Design and Information Technology Center and [b]Department of Chemistry, University of Toronto, Ontario, Canada**

Purpose: Design and synthesis of fluoropeptidomimetics (**1**) as potential irreversible inhibitors against serine proteases and their biological activity evaluations. **Methods**: The design of fluoropeptidomimetics as a new class of transition-state analogues is based on the hypothesis that enzymes bind tighter to their transition state than to their corresponding substrate or the product. Compounds such as **1** (X = NH or S) are designed based on the mechanism of hydrolysis of a peptide bond by serine or cysteine proteases. Towards the synthesis, biological activity studies and the stability studies of these compounds **2**-**9**, various fluorinating methods, spectroscopic tools were used. **Results**: α-Fluoro glycine derivative **2** and α-fluorothio derivatives **3**-**9** were synthesized for the first time *en route* to obtain the fluoropeptidomimetics such as **1**. Ac-

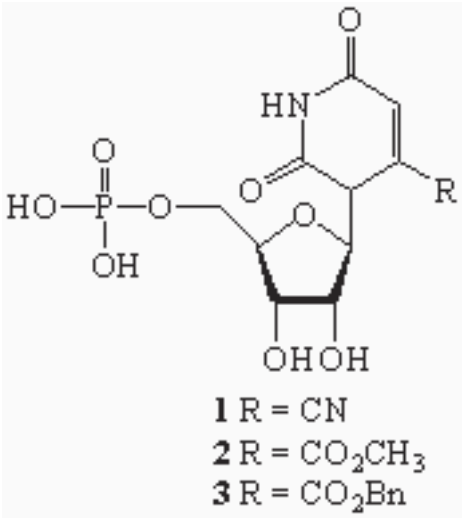
cordingly, various α-fluorothio monomers were prepared and coupled with another amino acid to get the target dipeptides **3**-**9**. Compound **8** was tested against α-chymotrypsin considering the large hydrophobic pocket at P1 residue. The compound **8** showed up to 30% inhibition at 2 h in a time-dependent assay. The stability of **2**-**9** in aqueous media and the stability of **3**-**9** in acidic and basic media were also studied. **Conclusion**: a new class of fluoropeptidomimetics were designed, compounds 2-9 were synthesized, and the biological activity of compound **8** was tested against α-chymotrypsin. Apart from the synthesis, the stability of these compounds in aqueous media, acidic as well as basic media will be presented.



11. DESIGN, SYNTHESIS AND X-RAY CRYSTAL STRUCTURES OF NUCLEOSIDE PROBES AGAINST OROTIDINE MONOPHOSPHATE DECARBOXYLASE (ODCASE)

***Subhash Annedi*[a,c,§], Masahiro Fujihashi[d,§], Lianhu Wei[a,c], Emil F. Pai*[c,d,e] and Lakshmi P. Kotra*[a,b,c].**
[§]Both authors contributed equally to this paper.
[a]Faculty of Pharmacy, [b]Department of Chemistry, [c]Molecular Design and Information Technology Center, University of Toronto, Ontario, Canada; [d]Division of Molecular and Structural Biology, Ontario Cancer Institute/University Health Network, [e]Departments of Biochemistry, Medical Biophysics, and Molecular & Medical Genetics, University of Toronto, Ontario, Canada

Purpose: Design and structural studies of orotidine 5'-monophosphate (OMP) derivatives against ODCase as potential antiviral agents. **Methods**: As part of our strategic drug discovery program, we synthesized various OMP derivatives (**1**-**3**) to probe the ligand binding properties of ODCase. Compound **1** was prepared from 5-bromouridine, and compounds **2** and **3** were prepared from uridine. Native ODCase was incubated with several equivalents of **1**, **2** or **3**, and crystallized according to published procedures. Diffraction data were collected at synchrotron sources and the structures of the three complexes were determined by molecular replacement techniques and refined using MD-refinement. **Results**: Compounds **2** and **3** are novel compounds against ODCase. Compound **2** binds in the active site of ODCase similar to compound **1**, indicating that the binding pocket does possess flexibility. Co-crystals of **3** and ODCase produced only a complex of UMP bound in the active site of ODCase implying that the benzyl carboxyl moiety has been eliminated, probably via chemical means. Details of the binding site and ligand binding properties in relation to novel drug design will be discussed in this presentation. **Conclusion**: The binding pocket of ODCase appears to be less rigid than originally thought, and these observations can be used in the design of novel drugs directed against RNA viruses.



12. INVESTIGATIONS ON THE MOLECULAR MECHANISMS OF HUMAN CYTOMEGALOVIRUS PROTEASE – ROAD TO THE DESIGN OF INHIBITORS

***Lianhu Wei,*[a] Liang Tong[c] and Lakshmi P. Kotra*[a,b],**
[a]Faculty of Pharmacy, and Molecular Design and Information Technology Center, [b]Department of Chemistry, University of Toronto, Ontario, Canada; [c]Department of Biochemistry, Columbia University,

New York, New York, USA

Purpose: Investigate the mechanism of human cytomegalovirus (HCMV) protease, its dimerization and design mechanism-based inhibitors. **Methods**: As part of our strategic drug discovery program, we are interested in understanding the dynamic nature of therapeutically important HCMV protease. The structures of HCMV protease either were obtained from the RCSB at Rutgers University or were determined at Columbia University. Molecular dynamics simulations and energy minimizations were performed using Amber 7.0 suite of programs. In separate simulations studies, monomeric and dimeric forms of HCMV protease were immersed in a periodic box of TIP3P water having a 10 Å thickness. The molecular systems were energy-minimized and molecular dynamics simulations were performed for approximately 6.5 ns on an Onyx or Origin supercomputer. **Results**: Snapshots of the protease at each pico second from the two MD simulations (inactive monomeric and the active dimeric form) were analyzed. Analyses of the MD trajectory including several x-ray crystal structures of the HCMV protease suggested significant structural differences in the dimeric interface in the dimmer form, and a similar region in the monomeric form of the protease. The interaction of áF in the dimer interface appears to be critical to the catalytic activity and to the stability of the protease. We also observed during the simulations that side chain of E105 in one of the monomers in the dimer may contribute to the S1' pocket formation, as well as interactions with the P1' residue, based on the dynamic nature of the residues and MD trajectory. Details on the simulations trajectories and implications towards inhibitor design will be discussed. **Conclusion**: A 6.5 ns molecular dynamics simulations on the monomeric and dimeric forms of HCMV protease provide an insight into some mechanistic aspects and dynamic nature of this enzyme.

13. NOVEL MECHANISM BASED INHIBITORS OF HUMAN CYTOMEGALOVIRUS PROTEASE: "HIS-BLOCK" STRATEGY

***Baskar Mannargudi*[1], Farooq A. Biabani[1], Subhash C. Annedi[1], Lianhu Wei[1], Liang Tong[2] and Lakshmi. P. Kotra[1]; [1]Department of Pharmaceutical Sciences and Molecular Design and Information Technology Center, University of Toronto, Ontario, Canada; [2]Department of Biological Sciences, Columbia University, New York, New York, USA**

Purpose: To design and synthesize tripeptide inhibitors and evaluate against human cytomegalovirus protease (HCMV) for the time dependent loss of activity. **Methods:** Tripeptide inhibitors were designed by molecular modelling which could interact with the catalytic machinery of HCMV protease and cause irreversible inhibition. These tripeptides contain D-serine, D-homoserine and D-hydroxy norvaline at P_1 position as the key moieties. The hydroxyl group on this D-amino acid at P_1 position is expected to interact with the catalytic His-63 through hydrogen bonding, thereby impairing the deacylation step causing irreversible inhibition. Five tripeptides containing the sequences AcNH-L-Val-L-Phe-D-Ser-NHBn (**1**), AcNH-L-Val-L-Phe-D-Hse-NHBn (**2**), AcNH-L-Val-L-Phe-L-Hse-NHBn (**3**), AcNH-L-Val-L-Phe-D-Nva (5-OH)-NHBn (**4**) and AcNH-L-Val-L-Phe-D-Ser-OMe (**5**) were designed for this purpose. **Results:** Compounds **1-5** were synthesized and biologically evaluated against chymotrypsin initially followed by HCMV protease. Compound **2** showed 31% and 43% enzyme inhibition against HCMV protease and chymotrypsin after 6 and 8 hours respectively. Other compounds did not show significant time dependent loss of activity. **Conclusion:** Five tripeptides were designed, synthesized and biologically evaluated against human cytomegalovirus protease. Compound **2** was chosen as the initial lead based on the biological results obtained to understand further structure activity relationships in these classes of compounds.

14. MODULATION OF ARYL HYDROCARBON RECEPTOR-REGULATED GENES BY TUMOR NECROSIS FACTOR-A AND LIPOPOLYSACCHARIDES.

***Cindy Lu*, Negar Gharavi and Ayman O. El-Kadi. Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada**

Purpose: The aims of the present study were to investigate the effect of the proinflammatory cytokine, tumor necrosis factor-a (TNF-a) and lipopolysaccharides (LPS) on the inducible expression of aryl hydrocarbon receptor- (AHR)-regulated genes; cytochrome P450 1a1 (Cyp1a1), glutathione S-transferse Ya (GST Ya), and NAD(P)H:quinone oxidoreductase (QOR). **Methods:** Murine hepatoma Hepa 1c1c7 cells were incubated with recombinant murine TNF-a (1, 5 and 10 ng/ ml of culture media) or LPS (1 and 5 mg/ml) in the presence of b-naphthoflavone (bNF, 10 mM). The cellular toxicity was determined using 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. At the activity level; 1) Cyp1a1 activity was measured using 7-ethoxyresorufin as a substrate, 2) cytosolic GST Ya catalytic activity was measured spectrophotometrically using cumene hydroperoxide as a substrate, and 3) cytosolic QOR activity was assayed spectrophotometrically as dicoumarol-inhibited 2,6-dichlorophenolindo-phenol reduction. At the RNA level, Cyp1a1, GST Ya and QOR mRNA were assessed by Northern blot analysis. **Results:** TNF-a and LPS had no apparent cellular toxicity effects on Hepa 1c1c7 cells at all concentrations tested. When cells were treated with the bNF alone, significant increases in the Cyp1a1, GST Ya and QOR were observed at the activity and mRNA level. When the cells were treated with bNF and TNF-a or bNF and LPS, the bNF-medi-

ated induction of Cyp1a1, GST Ya and QOR at activity level was significantly reduced in a dose-dependent manner by both TNF-a and LPS. In all cases, changes at the mRNA level paralleled changes at the activity level. **Conclusion:** TNF-a and LPS strongly repress the inducibility of Cyp1a1, GST Ya and QOR at the activity and mRNA level. In addition, TNF-a and LPS inhibit the Cyp1a1, GST Ya and QOR enzymes at the transcriptional level. **Acknowledgements:** NSERC and the Faculty of Pharmacy and Pharmaceutical Sciences at the University of Alberta supported this work. C. Lu was the recipient of the Merck National Summer Student Research Scholarship.

15. THE ROLE OF *HOXA2* ON THE EXPRESSION PATTERNS OF PAX6 AND OLIG2 AND ITS IMPACT ON OLIGODENDROCYTE DEVELOPMENT

***Kimberly Hogh*[1], Danette Nicolay[1], Ronald Doucette[2] and Adil Nazarali[1], [1]Laboratory of Molecular Biology, College of Pharmacy & Nutrition, [2]Department of Anatomy & Cell Biology, College of Medicine, University of Saskatchewan, Saskatoon Canada**

Purpose Although the spatial and temporal pattern of oligodendrocyte development in the embryonic spinal cord has been known for some time, the underlying molecular mechanisms controlling their progression from undifferentiated multipotent progenitors to mature myelinating oligodendrocytes has not been fully elucidated. *Hoxa2* gene is a transcriptional factor that has been demonstrated in our laboratory to be expressed in the oligodendroglial lineage. The objective of this investigation was to determine the potential influence of *Hoxa2* on the expression patterns of Pax6 and Olig2, two genes that have been implicated in oligodendrocyte development. **Methods** Immunohistochemical analyses were conducted to determine the dorsal-ventral distribution of Pax6 and Olig2 in mice spinal cords at embryonic ages E12, E14.5, and E17 in *Hoxa2* -/- and in *Hoxa2* +/- mice. **Results** Dorsal migration of Olig2, a gene whose expression is tightly linked to cells of the oligodendrocyte lineage after E12, appeared to be delayed in *Hoxa2* -/- mice at E14.5 only. However, when the dorsal-ventral cell counts of Olig2 were mapped in the spinal cords at E14.5, no significant difference was found between the knockout and heterozygous genotypes. Loss of *Hoxa2* gene appears to have no influence on Pax6 expression in mice spinal cords at all stages analyzed. **Conclusion** *Hoxa2* expression in oligodendrocyte lineage and its potential role in oligodendrogenesis may be downstream of *Pax6* and *Olig2*, since loss of *Hoxa2* does not appear to affect expression patterns of Pax6 and Olig2 in E12, E14.5, and E17 murine spinal cords. (Supported by CIHR and the MS Society of Canada. KH was supported by National Summer Student Research Program funded by the Merck Company Foundation).

16. IN VITRO AND IN VIVO EVALUATION OF SOFT ALKYLATING AGENTS: "CHLOROETHYLUREAS" AS POTENTIAL ANTICANCER DRUGS

***J. Fortin**, R. C.-Gaudreault°, E. Petitclerc°,*Faculté de pharmacie, Université Laval, Ste-Foy, Québec, Canada; °Centre de recherche, Unité des biotechnologies et de bioingénérie, C.H.U.Q., Hôpital St-François d'assise**

Purpose: In Canada, the incidence of cancer will have increased by about 70% in 2015. Cancer chemotherapy will require the discovery of new drugs having lower toxicity, larger therapeutic indexes and lower capacity to induce chemoresistance in tumor cells. To that end, a new class of antineoplastic agents is emerging. These agents are designated as ChloroEthylUreas (**CEU**). CEU are "soft" alkylating agents that do not alkylate DNA but are able to alkylate specific intracellular proteins such as ß-tubulin and other proteins involved in cellular motility. The inhibition of such proteins might have an impact in the treatment of important pathologies such as tumor proliferation and metastasis, arthritis, and psoriasis. **Methods and Results:** According to our experiments, a drug such as CEU-22 has been shown to abrogate the migration of endothelial cells (HUVEC) at concentrations ranging from 5 to 50µM. CEU-22 reduced by 50% the tumoral mass of CS1 cells (hamster melanoma tumour) in the CAM assay. The tumor masses were clearly depleted of blood vessels. Other CEU, such as CEU-98 and CEU-71 do not impede the migration of the HUVEC in the Boyden chamber assay. However, they still potent inhibitors of M21 (melanoma human) cell growth. The IC_{50} of these CEU are lower than cisplatin, which are an ADN polymerase inhibitor and a DNA cross-linker. For example, CEU-71, CEU-98 and cisplatin had IC_{50} of 6, 2 µM, 11, 3 µM, and18 µM, respectively. Moreover, we showed that the cytotoxycity of cisplatin is related to the nature of the matrix on which cells are growing. Such phenomenon is not observed with any of the CEU tested. The biochemical and the cellular mechanisms involved in this phenomenon are still unknown but there are most likely involved in tumor cell chemoresistance. **Conclusions:** Eventually our finding might help to design new potent anticancer drugs that will target specific and lethal biological pathways essential to tumor growth and dissemination. Moreover, these drugs might find application in several related pathologies. **Acknowledgements:** This work was supported by the Merck Company Foundation National Summer Student Research Program Abstract.

17. MODULATION OF CITRAL CYTOTOXICITY IN INTACT RAT HEPATOCYTES BY INHIBITING CARBONYL METABOLIZING ENZYMES OR MODIFYING CELLULAR REDOX POTENTIAL

***Francesca Ting-Yan Cheung* and Peter O'Brien; Department of Pharmaceutical Sciences, Faculty of**

Pharmacy, University of Toronto, Ontario, Canada

Background: Citral (3,7-dimethyl-2,6-octadienal) is an α,β–unsaturated aldehyde that occurs naturally in plants and citrus fruits. Due to its strong lemony aroma, it is widely used as an additive to food, cosmetics, and detergents. However, previous investigations have reported a variety of adverse effects associated with the use of citral. **Purpose**: This study investigated the toxicity of citral in intact rat hepatocytes. The effects of inhibiting carbonyl metabolizing enzymes or modulating cellular redox potential (by altering the free NADH to NAD^+ ratio or decreasing reduced glutathione levels) on hepatocyte susceptibility towards citral were examined to determine the role of different metabolizing enzymes in the detoxification of citral. **Method**: Hepatocytes isolated from male Sprague-Dawley rats were incubated with various compounds. Cell viability was assessed by plasma membrane disruption as determined by the trypan blue assay. Hepatocyte lipid peroxidation was determined by measuring the amount of thiobarbituric acid reactive substances (TBARS) formed by following the absorbance at 532 nm in spectrophotometer. **Results**: 0.85±0.05 mM of citral was required to lyse 50% of the hepatocytes (LD_{50}) after a 2-hour incubation. While NADH generators prevented cytotoxicity, NAD^+ generators had different effects on citral cytotoxicity depending on the location of the aldehyde dehydrogenase (ALDH). All ALDH inhibitors increased cytotoxicity. Citral cytotoxicity was prevented by the mitochondrial permeability transition inhibitor, carnitine, and by the glycolytic/citric acid cycle substrate, oxaloacetate. **Conclusion**: The oxidative detoxification of citral is compromised, as it is both a substrate and an inhibitor of ALDH. Citral was a better substrate for mitochondrial ALDH than cytosolic ALDH and citral cytotoxicity was significantly decreased by mitochondrial ALDH inhibitors. The metabolism of citral was similar to *trans,trans*-2,4-decadienal presumably because of their structural similarity but citral was less toxic than decadienal because citral is a poorer electrophile. **Acknowledgement**: This research was supported by the National Summer Student Research Program funded by the Merck Company Foundation.

18. EFFECT OF TRANS-RESVERATROL ON CYTOCHROME P450 1B1 GENE EXPRESSION IN MCF-7 HUMAN BREAST CARCINOMA CELLS AS DETERMINED BY REAL-TIME POLYMERASE CHAIN REACTION

Catherine Cheung **and Thomas K. H. Chang, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada**

Purpose. To determine the effect of *trans*-resveratrol on CYP1B1 gene expression in cultured MCF-7 human breast carcinoma cells. **Methods.** MCF-7 cells were cultured in Dulbecco's modified Eagle medium supplemented with 100 U/ml penicillin, 2 mM glutamine, 100 mg/ml streptomycin, and 10% fetal bovine serum. They were incubated at 37°C in a humidified, 95% air / 5% CO_2 atmosphere. Cells in the exponential growth phase were treated with *trans*-resveratrol (2.5, 5, 10, or 20 mM) or dimethylsulfoxide (0.5%, vehicle control) for 6 hours. Total cellular RNA was isolated and reverse transcribed. CYP1B1 cDNA was amplified by a real-time DNA thermal cycler (LightCycler®, Roche Diagnostics). The conditions were initial denaturation at 95°C for 5 min, followed by cycling at 94°C for 5 s (denaturation), 65°C for 10 s (annealing), and 72°C for 15 s (extension). Sequences for the forward (5'-CAC-TGC-CAA-CAC-CTC-TGT-CTT-3') and reverse (5'-CAA-GGA-GCT-CCA-TGG-ACT-CT-3') primers for CYP1B1 were synthesized at the UBC Nucleic Acid and Protein Service Unit. Each 20 ml reaction contained 1X PCR buffer, 4 mM $MgCl_2$, 0.25 mg/ml bovine serum albumin, 0.2 mM dNTP, 0.2 mM primers, 1:30,000 SYBR Green I, 0.05 U/ml *Taq* enzyme, and known amounts of cDNA. CYP1B1 amplicons were visualized by agarose gels stained with ethidium bromide. **Results.** Initial experiments were performed to validate our real-time polymerase chain reaction (PCR) method for the analysis of CYP1B1 gene expression. The standard curve was linear from 100 to 1,000,000 copies ($r^2 > 0.98$). The limit of quantitation was at least 100 copies. The intra-day variability (N = 4) and inter-day (N = 4) variability was <3% and <15%, respectively. *Trans*-resveratrol decreased CYP1B1 gene expression in MCF-7 cells, with an IC_{50} value of approximately 10 mM. **Conclusion.** *Trans*-resveratrol suppressed CYP1B1 gene expression in cultured MCF-7 human breast carcinoma cells, as determined by a validated, real-time PCR method. **Acknowledgements.** This research was supported by the National Summer Student Research Program funded by the Merck Company Foundation.

19. THE EFFECT OF VALPROIC ACID ON BIOMARKERS OF OXIDATIVE STRESS IN RATS

Frank Abbott**, Vincent Tong, and Thomas K.H. Chang, Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, Canada**

Recently, we demonstrated an association in rats between valproic acid (VPA) treatment and an increase in plasma levels of 8-isoprostane (8-iso-PGF_{2a}), which is used as an *in vivo* indicator of oxidative stress. This increase by VPA is enhanced by phenobarbital (PB) pretreatment, a known inducer of cytochrome P450-mediated VPA biotransformation. We propose that oxidative stress may be involved in VPA-induced hepatotoxicity. **Objectives**. To further demonstrate the association between VPA treatment and increases in oxidative stress using a variety of biomarkers and to investigate whether increases in these biomarkers in the liver reflect those observed in plasma. **Methods.** Sprague-Dawley rats (n=6/group) with or without pretreatment with PB (80 mg/kg/day for 3 days, ip) were

subsequently treated with VPA (500 mg/kg, i.p.) and sacrificed 0.5 hours later. Plasma and livers of rats were obtained for determination of free and total 8-iso-PGF_{2a}, hydroperoxide, and TBARS levels. **Summary**. Consistent with previous findings, levels of free 8-iso-PGF_{2a} were enhanced in VPA treated animals (69 ± 4 pg/mL) and further enhanced by PB pretreatment (130 ± 4 pg/mL) compared to control values (36 ± 4 pg/mL). Free 8-iso-PGF_{2a} levels in the liver were reflective of those observed in plasma. No differences were observed in levels of total 8-iso-PGF_{2a}, TBARS, and hydroperoxides measured in plasma and liver among the treatment groups. Our results confirm the greater sensitivity of 8-iso-PGF_{2a} as a biomarker for oxidative stress in plasma and liver in rats. Presented at the 11th North American ISSX meeting, October 27-31, 2002 in Orlando Fl. Published in Drug Metabolism Reviews, 34, 98 (2002).

20. EFFECTS OF CELECOXIB ON KIDNEY FUNCTION.

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Purpose: The present study examines the effects of celecoxib, a COX-2 inhibitor, on renal function of the rat. The objective of this study is to compare plasma concentrations of celecoxib to urine volume and electrolyte excretion rates. We expected a relationship between celecoxib plasma concentration and its renal effect reflected by electrolyte excretion abnormality. Our hypothesis is that potassium and sodium excretion rates, as well as urine volume, will diminish as plasma trough concentration of celecoxib increase. **Methods:** Adult male Sprague-Dawley rats (350-450g) were administered single doses of celecoxib by oral gavage at 40mg/kg for four consecutive days. Immediately after dosing, the rats were housed in metabolic cages and urine output was collected during 0-12 hours. Overnight, rats were given free access to food and water. Blood samples were collected by tail vein extraction at 8 and 12 hours post-dose on day 4. **Results:** There was a significant reduction in urine volume (-25.79%, $p<0.05$), sodium excretion rate (-35.41%, $p<0.05$) and potassium excretion rate (-36.20%, $p<0.05$) by rats receiving celecoxib as compared to controls. Significant correlation was found between 8 hour plasma celecoxib concentration and 12 hour urine volume ($r=0.7829$, $p<0.05$), as well as 8 hour plasma celecoxib concentration and 12 hour average sodium excretion rate ($r=0.8249$, $p<0.05$). No correlation was observed between plasma celecoxib concentration and potassium excretion rate. **Conclusion:** Celecoxib plasma concentrations appear to affect sodium urine excretion rate and urine volume. JMG was a recipient of a Merck-Frosst Summer Research Scholarship.

21. THE ROLE OF P-GLYCOPROTEIN IN THE PHARMACODYNAMICS OF VERAPAMIL.

***Yaman Dakhel* and Fakhreddin Jamali, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada**

Purpose. Patients with rheumatoid arthritis and rats treated with interferon-a2a have reduced dromatropic response to verapamil, measured as PR-interval prolongation. This is due, likely, to a receptor down-regulation. We hypothesized that P-glycoprotein, which may be increased due to inflammation, limits access of the drug to the target protein. Both verapamil enantiomers inhibit P-glycoprotein; however, R has much less dromatropic effect. **Method**. Dromotropic activity of verapamil was measured in normal and interferon-treated rats following the administration of i.v. doses of a racematic (1 mg/kg) or a non-racemic verapamil formulation (0.3 mg/kg S and 2.1 mg/kg R). The two doses had equal dromotropic effects but the non-racemic possesses much greater P-glycoprotein inhibitory effect. Inflammation was induced by s.c. injections of two doses of $5.0x10^4$ units of interferon-a2a 12 and 3 h prior to verapamil administration. Segmented neutrophils count was used as a measure if inflammation. Metal electrodes were implanted into the right and left axilla and electrocardiogram was recorded at the baseline and up to three hours after verapamil administration. **Results**. Inflammation reduced response to verapamil. There was no significant difference between racemic and non-racemic treatments. **Conclusion**. Greater doses of P-glycoprotein inhibitor did not influence the inflammation-induced reduced response to verapamil. This indirect evidence suggests that P-glycoprotein has no significant role in the effect of inflammation on cardiovascular receptors. Supported by CIHR.

	Racemic		Non-racemic	
	Normal	Inflamed	Normal	Inflamed
% Segmented neutrophils	2.4 ± 0.9	12.2 ± 2.9[a]	2.3 ± 0.7	13.6 ± 2.5[a]
PR interval, % maximum change	16.6 ± 6.6	3.6 ± 3.6[a]	14.8 ± 6.6	5.4 ± 3.4[a]
PR interval, $AUEC_{0-30}$ (min)	180 ± 72	30 ± 38[a]	217 ± 44	82 ± 41[a]

[a] significantly different from Normal; N= 4 or 5/group. $AUEC_{0-30}$, area under effect-time curve 0-30 min post dose.

22. INTERACTION BETWEEN ERYTHROMYCIN AND VERAPAMIL.

***Yaman Dakhel* and Fakhreddin Jamali, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Canada**

Purpose. A case report suggests that co-administration of verapamil and erythromycin results in complete artioventricular (AV) block and QT interval prolongation. Using the rat as an animal model, we investigated this potential drug interaction. **Methods**. Three groups of adult male Sprague Dawley rats (280-300 g) were studied: Group I received 1 mg/kg intravenous verapamil; Group II received 100 mg/kg intravenous

erythromycin; Group III received first 100 mg/kg intravenous erythromycin and 10 minutes later 1 mg/kg intravenous verapamil. Metal electrodes were implanted into the right and left axilla and electrocardiogram was recorded at the baseline and up to five hours post dose. **Results**. Verapamil and erythromycin, when given alone, significantly prolonged PR and QT intervals, respectively. When given together, a more than a 2-fold increase in prolongation of PR interval and an 83% incidence of second-degree AV block were noticed. Therefore, the combination of erythromycin and verapamil caused arrhythmia and synergetic effect on PR-interval prolongation but not on QT interval. **Conclusion**. There was a significant interaction between intravenous verapamil and erythromycin resulting in arrhythmia and a synergetic effect on PR-interval prolongation. This confirms the anecdotal observation in humans. Supported by CIHR.

Cardiovascular indices	Verapamil (n = 6)	Erythromycin (n = 6)	Erythromycin + Verapamil (n = 6)
PR-interval	18.3 ± 7.4[a]	No Change	38.4 ± 19.1[a,b]
QT interval	No Change	96.2 ± 12.8[a]	98.4 ± 14.5[a]
Arrhythmia incidence	0/6	0/6	5/6[a,b]

[a] Significantly different from baseline; [b] Significantly different from other groups.

PR and QT intervals are expressed as the percent of maximum change from baseline.

23. THE EFFECT OF INFLAMMATION ON THE BINDING OF CARDIOACTIVE AGENTS TO RAT CARDIAC CELL MEMBRANE.

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Purpose. Inflammation attenuates the response to cardiovascular drugs. The purpose of this work was to investigate the effect of inflammation on the binding of radioligands to their cardiac receptors. **Methods.** Chronic and acute inflammation states were induced either by single injection of 10 mg killed *mycobacterium butyricum* into the tail base (adjuvant arthritis) or by two subcutaneous injections of $5x10^4$ unit interferon-a2a over 8 h, respectively. Three weeks after induction of adjuvant arthritis and 4 h after the second injection of interferon, the hearts were removed, homogenized and centrifuged to provide cell membranes. Equilibrium ligand binding studies were performed using ^{3}H-nitrendipine (NIT) or ^{3}H-dihydroalprenolol (DHA). Equilibrium bindings were also performed on normal rat cardiac membranes in the presence of TNF-a. The binding parameters, dissociation constant (K_D) and maximum binding (B_{max}) were determined. **Results.** Results indicate little change in K_D values, but B_{max} for NIT in chronic and acute inflammation was significantly reduced by 38.7 ± 6.8% and 26.1 ± 8.1%, respectively. B_{max} for DHA was also significantly reduced by 42.7 ± 10.1% and 20.3 ± 8.7% ($p< 0.05$). Pre-incubation of TNF-a did not affect the binding parameters of either ligand to normal membranes. **Conclusion.** The attenuation of cardioactive drug efficacy seen in inflamed states is due to, at least in part, the reduction in the available binding sites on cardiac myocytes. Supported by the Canadian Institute for Health Research.

24. THE EFFECT OF INTERFERON-INDUCED INFLAMMATION ON THE TIME COURSE OF INFLAMMATORY MEDIATORS, TNF AND NO IN THE RAT

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Purpose. Tissue necrosing factor-á (TNF) and Nitric oxide (NO) are believed to play important role in pathogenesis of inflammatory diseases. The purpose of this work was to study the time course of TNF and NO in plasma after injection of interferon-á2a (INF) to rats. **Methods.** Male Sprague Dawley rats (300 ± 20 g, n=8) were cannulated via right jugular vein. After overnight recovery, animals were divided into two groups of control and inflamed rats. Just prior to the commencement of blood sampling and 8 h after, 0.2 ml of either sterile saline (control) or 50000 U of INF (inflamed) were subcutaneously injected. Blood samples were collected for 72 h. Plasma was harvested and kept at – 70 until analyzed to measure concentrations of TNF and NO using ELISA kit and colorimetric method, respectively. **Results.** Area under the time-plasma concentration (AUC) of TNF was significantly raised from 1704 ± 468 pg/mL.h in controls to 4968-± 449 pg/mL.h in inflamed rats. Similarly, the AUC of NO was significantly higher in inflamed (1405 ± 522 ìM) compared with control (4486 ± 461 ìM) rats. The rise in NO lagged that of TNF for few hours. The concentration of TNF returned to normal after 72 hours, however the concentration of NO remained higher than normal values. **Conclusion.** INF-induced acute inflammation is associated with increased production of TNF and NO. The production of NO may be triggered by TNF due to expression of inducible nitric oxide synthase (iNOS). Supported by the Canadian Institute for Health Research (CIHR).

25. PHARMACOKINETIC STUDY OF MELOXICAM IN THE RAT

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Purpose: Meloxicam is a cyclooxigenase II selective non-steroidal anti-inflammatory drug (NSAID) belonging to enolic acid class. Pharmacokinetics of meloxicam has been investi-

gated in a number of animal species using oral solutions of the drug with an absolute bioavailability range of 90-99%. Meloxicam, however, has a very low water solubility hence its bioavailability from solid dosage form is expected to be less than that reported for solutions. The aim of this study was to determine the pharmacokinetics particularly the absolute bioavailability of meloxicam after oral administration of its solid dosage form to the rat. **Method:** Adult male Sprague-Dawley rats were cannulated in the right jugular vein and were allowed to recover overnight. They were divided to two groups. Both groups received single doses of 840 µg/kg of meloxicam, either via iv (dissolved in 1 mL 5 mM NaOH injected over 1 min, n=5) or oral (crushed tablet of commercially available tablet using a plastic gavage tube, n=6) routes. Overnight before dosing until 4 h post-dose, the animals were deprived of food but had free access to water. Serial blood sample were collected over 0-48 h post dose, and plasma meloxicam concentration was quantified using a validated HPLC assay. Pharmacokinetic indices were calculated using the model-independent, non-compartmental method. **Results**: The absolute bioavailability of the oral meloxicam was 0.64. **Conclusion:** Meloxicam bioavailability from the available dosage form is incomplete.

	C_{max} µg/mL	T_{max} h	$t_{1/2}$ h	AUC_{0-48} µg.h/mL	$AUC_{0-\infty}$ µg.h/mL	V_d L/kg	CL L.kg/h
iv	nd	nd	32.7 (27.5)	80.7 (19.0)	132 (63.9)	0.28 (0.11)	9.0 (4.1)
po	2.3 (0.9)	8.4 (5.0)	20.9 (5.2)	53.8 (35.2)	85.1(32.9)	0.21 (.071)	7.0 (3.0)

nd, not determined; values are mean±SD.

26. INFLUENCE OF FCA-MEDIATED INFLAMMATION ON THE METABOLISM OF MORPHINE IN RAT LIVER MICROSOMES: IMPLICATIONS FOR MORPHINE PHARMACOKINETICS

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Purpose. Some rodent pain models of persistent inflammation rely on the administration of Freund's Complete Adjuvant (FCA) to create hyperalgesic/allodynic conditions. Since little is known about the influence of the acute inflammatory reaction on the pharmacokinetics of analgesics, we evaluated the metabolism and pharmacokinetics of morphine (M) in naïve and FCA-treated rats using *in vitro* and *in vivo* approaches. **Methods.** *In vitro studies*: Livers from rats treated with a single sub-dermal FCA injection (100 µg) were collected 24h later. Naïve rats were used as controls. Microsomes were prepared from both groups. Following incubation of M in liver microsomes, morphine-3-glucuronide (M3G) and normorphine (NM) formation were measured. *In vivo studies*: M pharmacokinetics were investigated in naïve and FCA-treated rats after intravenous (10 µmol/kg) or oral (50 µmol/kg) administration. In all studies, HPLC-MS was used to measure M, M3G and NM. **Results.** *In vitro*, NM formation was significantly decreased (46 % of control) in liver microsomes from FCA-treated rats compared to naïve rats, whereas the formation of M3G remained unchanged. Similarly, *in vivo*, NM formation, expressed as metabolite/parent AUC ratios, significantly decreased in FCA-treated animals (10-44 % of control). The M3G/M AUC ratios were comparable regardless of FCA administration. The pharmacokinetics of M, administered intravenously or orally, was not significantly different between naïve and FCA-treated rats. **Conclusion.** CYP-mediated NM formation appears more sensitive to the inflammatory stimulus than M3G formation. Since NM is a minor metabolite than M3G, this did not have an impact on the pharmacokinetics on M in FCA-treated rats.

27. EFFECT OF DILTIAZEM AND LOSARTAN ON RBC ADENINE NUCLEOTIDES *CONCENTRATIONS IN VIVO*

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Purpose. To determine the effect of diltiazem (DTZ) and losartan on RBC concentrations of ATP, ADP and AMP using an *in vivo* rat model. **Methods.** Male Sprague Dawley (SD) rats were used for the experiment (n= 4 - 8). Prior to the experiment, a polyethylene catheter was implanted into the right carotid artery of each rat under general anaesthesia and exteriorized for blood sample collection. After recovery from the surgery (24-h), a blood sample (1 mL) was collected from each animal into a polyethylene microcentrifuge tube containing 0.15 mL of a "Stopping Solution" to prevent in vitro formation and degradation of adenosine and adenine nucleotides. Each animal then received 10 mg/kg diltiazem or losartan s.c. bid for five doses. A blood sample (1 mL each) was obtained 1h after the last dose. RBC concentrations of adenine nucleotides (ATP, ADP and AMP) were determined by a previously reported HPLC. **Results.** DTZ increases RBC concentrations of ATP (1.2 vs. 3.2 mM), ADP (1.3 vs. 1.6 mM), and AMP (13 vs. 16 mM). The effect of losartan was minimal (ATP 4.9 vs. 4.6 mM; ADP 2.8 vs. 3.2 mM; AMP 2.7 vs. 1.2). There were large differences of the data obtained between the rats. **Conclusion.** DTZ increases RBC concentrations of adenine nucleotides in rats, whereas the effect of losartan was considered minimal. RBC nucleotide concentrations could be used as a therapeutic marker to assess the *in vivo* action of anti-ischemic drugs in pre-clinical studies.

28. INDUCTION OF MRP3 BY ACTIVATORS OF THE PREGNANE X RECEPTOR

Shirley Teng **and Micheline Piquette-Miller, Department of Pharmaceutical Sciences, University of Toronto, Ontario, Canada**

Purpose. The pregnane X receptor (PXR) has been shown to be involved in mediating the induction of the MDR1, MRP2 and OATP2 transporters by xenobiotics. Furthermore, PXR activators induce MRP1 and MRP2 expression in human cell lines. Our studies were aimed at determining the involvement of PXR in the in vitro and in vivo regulation of MRP3 expression. **Methods**. The human hepatoma cell lines HuH7 and HepG2 were treated for 6 to 24h with PXR activators including clotrimazole, rifampicin, RU486, PCN, nifedipine, lithocholic acid or metyrapone. Levels of mRNA of MRP3, MRP2, MRP6 and PXR were measured by RT-PCR. MRP functional activity was determined by measuring the efflux of 5-carboxyfluorescein. In addition, mice were treated with RU486 for 3 days and hepatic transporter mRNA levels were measured by RT-PCR. **Results**. MRP3 mRNA levels in HuH7 cells were induced 1.6- to 8-fold in a dose- and time-dependent manner following treatment with the PXR activators whereas MRP6 expression was not affected. MRP functional activity in PXR activator-treated cells was induced to an extent corresponding with MRP3 mRNA induction. MRP3 and MRP2 mRNA levels were also induced in HepG2 cells. Furthermore, hepatic MRP3, MRP2 and PXR mRNA levels were induced 1.5-fold in RU486-treated mice. **Conclusions**. These results suggest that PXR activation may play a role in the regulation of MRP3 and MRP2 expression but not that of MRP6.

29. A NOVEL CIS-ACTING ELEMENT IS INVOLVED IN TNF-α MEDIATED PROMOTER ACTIVITY OF THE RAT MDR1B

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Purpose. Acute inflammation induced by cytokines or lipopolysaccharide (LPS) have been shown to decrease basal expression of the multidrug resistance gene, *mdr1b* as well as suppress its induction in the liver and cultured hepatocytes of rats. As the *mdr1b* promoter region between nt –291 and -278 contains several putative binding sites for C/EBPβ (NF-IL6) or STAT3, we hypothesize that binding of negative regulatory transcription factors to this region may be involved in the cytokine-mediated downregulation of *mdr1b* transcription. **Methods.** Male Sprague-Dawley rats were administered 5mg/kg LPS i.p. or saline. Livers were isolated at 2, 4, 6, 12, and 24 hrs after LPS treatment and hepatic nuclear protein were isolated. Electrophoretic mobility shift assay (EMSA) of ^{32}P-radiolabelled mdr1b promoter fragment and hepatic nuclear protein extracts was performed. The impact of 24 hr cytokine treatments on chloramphenicol acetyltransferase (CAT)-promoter fragment construct activity was examined. **Results.** EMSA showed that nuclear extracts from livers of rats treated with LPS formed a protein complex with the mdr1b promoter fragment (-449 to 111). Competition and supershift experiments suggested promoter fragment binding to NF-IL6 and STAT3. CAT reporter assays indicated a dose-dependent IL-6 mediated induction of transcription in both wildtype (-449mdrWT-CAT) and deletion constructs (-449mdrDEL1-CAT). In contrast, TNF-α mediated down-regulation of transcription occurred only in promoter fragments in which the putative site (-291 to -278) was deleted. **Conclusions.** The mdr1b promoter region between -291 to -278 appears to have putative binding sites for NF-IL6 and STAT3. When this region is deleted, TNF-α but not IL-6 down-regulates transcriptional activity. This site may play a role in mediating the suppression of mdr1b induction during acute inflammation previously reported by our laboratory. Further studies are required to confirm which of these transcription factors negatively regulates the mdr1b promoter via TNF-α.

30. EXPRESSION OF *MDR1A*/PGP IN RAT BRAIN DURING ENDOTOXIN-INDUCED SYSTEMIC INFLAMMATION

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Purpose. Exposure of animals to endotoxin (LPS) is known to cause reductions in hepatic and intestinal expression of P-glycoprotein (PGP)/*mdr1a*. As PGP also plays a critical role in drug distribution into the brain, we investigated the effects of LPS treatment on the expression and functional activity of PGP/*mdr1a* in rat brain. **Methods**. Male Sprague Dawley rats (250-275 g, n=4/group) were administered 5 mg/kg LPS i.p. Controls received saline. Rats were sacrificed at 6, 12 and 24 hrs after dosing, and the whole brain was collected for total RNA and isolation of membrane proteins. Levels of *mdr1a* and *mrp1* mRNA were measured by RT-PCR and normalized to *GAPDH*. PGP protein levels were detected on Western blot using C219 antibody. For determining *in vivo* activity, ^{99m}Tc-sestamibi [^{99m}Tc-MIBI, 20 MBq], a substrate of PGP, was administered via tail vein at five hrs of treatment, and whole body imaging were performed at various times. One and two hour wash out efflux rates of ^{99m}Tc-MIBI from brain were calculated. **Results**. As compared to controls, levels of *mdr1a* mRNA in cerebrum were decreased to 48 % and 54 % after 6 and 12 hrs of LPS treatment, respectively ($p<0.05$). Levels returned to 96 % after 24 hrs. A downregulation in the immunodetectable levels of PGP were seen in LPS treated rats. As compared to controls, radioimaging studies demonstrated substantially reduced rates of ^{99m}Tc-MIBI efflux from brain in LPS treated rats. Levels of mrp1 were not significantly affected. **Conclusions**. Our results indicate that LPS-

induced systemic inflammation generates a significant but transient down-regulation of *mdr1a* mRNA expression in rat cerebrum. A corresponding downregulation in PGP protein expression and efflux activity occurred after LPS treatment. Hence, infection or acute inflammation is likely to impose transient changes in brain disposition of PGP substrates.

31. INTERLEUKIN-6 DOWNREGULATES THE EXPRESSION OF PREGNANE X RECEPTOR (PXR) IN MICE.

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Purpose. The nuclear pregnane X receptor (PXR) mediates the transcriptional activation of several drug metabolizing enzymes and efflux transporters. Several of these enzymes and drug transporters have previously been shown to be downregulated in rodent liver during acute inflammation. Thus, we examined the effect of endotoxin and cytokine-induced inflammation on the expression of PXR as well as several drug transporters (*Oatp2, Mdr1b, Mrp2* and *Mrp3*) in mice. **Methods.** Male CD-1 mice (n = 4-6/group) were dosed i.p. with endotoxin (LPS, 5 mg/kg), interleukin (IL)-1b, IL-6, or tumor necrosis factor (TNF)-a. Levels of PXR, *Oatp2, Mdr1b, Mrp2* and *Mrp3* mRNA were determined by RT-PCR in livers collected at six or 24 hr. **Results.** Exposure of mice to endotoxin resulted in significant reductions in mRNA levels of PXR (32 ± 15% controls, $p<0.05$), associated with marked downregulation of *Oatp2, Mdr1b, Mrp2* and *Mrp3* mRNA expression (30%, 55%, 15% and 60% controls, respectively). IL-6 at a dose of 10,000 units also caused suppression in mRNA levels of PXR (48 ± 12% controls, $p<0.05$), as well as *Oatp2, Mdr1b, Mrp2* and *Mrp3* (20%, 32%, 35% and 60% of controls, respectively). Administration of TNF-a or IL-1b had no significant effects on levels of PXR, and caused only minor changes in transporter mRNA expression. **Conclusion.** Our data demonstrated that endotoxin and IL-6 significantly decreased the hepatic expression of PXR in mice. Regulation through PXR may provide a plausible mechanism for the downregulation of drug transporters during the acute phase response.

32. THE EFFECTS OF FM-VP4, A WATER-SOLUBLE PHYTOSTANOL, ON CHOLESTERYL ESTER TRANSFER PROTEIN (CETP) MEDIATED TRANSFER OF CHOLESTERYL ESTERS (CE) BETWEEN HIGH- AND LOW-DENSITY LIPOPROTEINS.

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Purpose: The purpose of this study was to determine if FM-VP4, a novel water-soluble phytostanol, modifies CETP-mediated transfer of CE between high-density (HDL) and low-density (LDL) lipoproteins. **Methods:** Experimental strategies involving the supplementation and inhibition of CETP were used to test the hypothesis that FM-VP4 would inhibit CETP-mediated CE transfer between lipoproteins. To determined if FM-VP4 modified CE transfer [^{3}H]cholesteryl oleate (^{3}H-CE) enriched LDL was co-incubated with increasing concentrations of FM-VP4 (10-100 uM) in T150 buffer (50 mM Tris-HCl, 150 mM NaCl, 0.02% sodium azide, 0.01% disodium EDTA), pH 7.4 which contained unlabeled HDL ± purified CETP (4 ug protein/ml) or in delipidated plasma which contained endogenous CETP (1-2 ug protein/ml) for 90 minutes at 37^0 C. Following incubation, LDL was precipitated and the precent of CE transferred over 90 minutes was determined. TP2 (4 ug protein/ml), a monoclonal antibody directed against CETP, and deoxycholate (50 uM), a known surface active agent, were additional treatment groups co-incubated with the radiolabeled LDL. TP2 was used to ensure the CE transfer measured was CETP-mediated and deoxycholate was used to demonstrate the observed effects of FM-VP4 were not purely a surface-active phenomenon. **Results:** FM-VP4 significantly decreased the percent transfer of ^{3}H-CE from LDL to HDL in a concentration-dependent manner following co-incubation with purified or delipidated plasma CETP. TP2 at 4 ug protein/ml significantly decreased the percent transfer of ^{3}H-CE from LDL to HDL following co-incubation with purified or delipidated plasma CETP. However, co-incubation with Deoxycholate resulted in no significant differences in ^{3}H-CE percent transfer compared to untreated controls. **Conclusions:** These findings suggest that FM-VP4 significantly decrease CETP-mediated transfer of CE from LDL to HDL. **Acknowledgements:** Canadian Institutes of Health Research (CIHR)-Forbes Medi-Tech Inc. University/Industry Operating Grant

33. THE ROLE OF LOW-DENSITY LIPOPROTEIN RECEPTORS (LDLR) AS A POSSIBLE UPTAKE MECHANISM OF CYCLOSPORIN A (CSA) INTO LLC-PK$_1$ PIG KIDNEY CELLS.

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Purpose: Previous studies in our laboratory have suggested that elevated LDL-cholesterol levels decreased cyclosporine A-induced cytotoxicity and uptake in LLC-PK1 cells, a pig renal epithelial cell line. In this study, we investigated the potential role of low-density lipoprotein receptors (LDLr) in the uptake of cyclosporine A (CsA) within the same cell line. **Methods**: A purified monoclonal antibody (IgG-C7) directed against human LDLr from a mouse was chosen to specifically bind to the LDLr expressed in LLC-PK1 cells. Cells were cultured at 37o C hu-

midified 5% CO2 in DMEM:Ham's F12 (1:1) medium containing 10% fetal calf serum supplemented with 100 U/ml penicillin and 100 mg/mL streptomycin. Once cells had grown to 90% confluence in a 96-well plate (seeding density at 7X104 cells/cm2), three treatment groups consisting of **A)** [3H] CsA alone; **B)** complex [3H] CsA-LDL; and **C)** [3H] CsA + LDL co-addition were pre-incubated in the presence and absence of 0.5ug/ml of IgG-C7 for 6 hrs. Following, the wells were spiked with 800ng/ml of [3H] CsA with and without 20ug/ml of LDL for an additional 24 hrs. Samples were analyzed for intracellular radioactivity. **Results**: A significant result was observed in **A)** [3H] CsA alone treatment group with the Student t-test ($p < 0.05$ vs. absence of IgG-C7). However, no significance was observed in both the **B)** complex [3H] CsA-LDL and **C)** [3H] CsA + LDL co-addition treatment groups. **Conclusions:** These results suggest that LDL receptors are playing a role in the uptake mechanism of CsA. IgG-C7 can significantly reduce CsA uptake in LLC-PK1 cells; yet when LDL is present, there is no significance. It is possible that CsA has preferential association with LDL and is taken up via a non-specific LDL receptor pathway, and/or CsA may be taken up via the LDL receptor independent of its association with LDL. **Acknowledgements:** Canadian Institutes of Health Research Grant (#MOP-1448)

34. THE INFLUENCE OF EXPERIMENTAL SCHISTOSOMIASIS ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF PHENOBARBITAL AND DIPHENYLHYDANTOIN IN MICE

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Absent

35. CELLULAR SIGNALING MECHANISMS OF CB1 CANNABINOID RECEPTORS

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Purpose: CB1 cannabinoid receptors in the brain are coupled to the Gi/Go family of G protein heterotrimers (alpha, beta and gamma subunits). Their cellular signaling mechanism, however, are poorly understood. **Methods:** CB1 cannabinoid receptors are expressed on N18TG2 neuroblastoma and C6 glioma cells. Extracellular signal-regulated kinases 1 and 2 (ERK1/2) are a group of kinases that play an important role in cell proliferation and differentiation. Using N18TG2 neuroblastoma and C6 glioma cells, we investigated the influence of CB1 cannabinoid agonists on the ERK pathway. N18TG2 cells C6 glioma cells were serum-starved (12- 24 h) and treated with cannabinoid receptor antagonist SR141716 prior to stimulation. **Results:** Treatment of cells with CP 55,940, Win 55212-2 or methanandamide stimulated phosphorylation of ERK. The stimulation of ERK was quantitated by densitometry of bands on Western blots of cell lysates. All drugs produced a concentration-dependent increase in the level of the phosphorylated forms of ERK. Studying the time-course for such stimulation revealed that the maximum effect was obtained 2 minutes of treatment with different CB1 agonists. The influence of pretreatment with pertussis toxin on this signaling pathway was also investigated. **Conclusions:** These studies elucidate a signal transduction relay between CB1 receptor activation of G proteins and the ERK pathway in cells. Supported by NIDA grant R01-DA03690 to AH and NINDS grant F05-NS11110 to AA.

36. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR COMMON SUNSCREEN AGENTS: APPLICATION TO *IN VIVO* ASSESSMENT OF SKIN PENETRATION AND SYSTEMIC ABSORPTION IN HUMAN VOLUNTEERS.

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Purpose: To develop a reverse-phase high-performance liquid chromatographic assay for quantifying four common sunscreen agents, namely 2-hydroxy-4- methoxybenzophenone (oxybenzone), 2-ethylhexyl-p-methoxycinnamate (octylmethoxycinnamate), 2-ethylhexyl-salicylate (octylsalicylate), and salicylic acid 3,3,5-trimethylcyclohexyl ester (homosalate) in a range of biological matrices. Skin penetration and systemic absorption of sunscreen filters after topical application to human volunteers were also measured. **Methods:** Separation was achieved utilizing a Symmetry C-18 column with methanol-water as the mobile phase. A preliminary study involved application of Coppertone Colorblok^{O} for kids (SPF 30) on arms and back of the volunteers. This commercially available sunscreen product contained the above four sunscreen filters. Sunscreen content in the stratum corneum was measured using the tape-stripping technique at 30 min, 4 and 8 hours. Blood samples were taken from all subjects at pre-application and at 1, 2, 4, 6, 8 and 24 hours post-application. The volunteers collected urine samples for 48 hours after application, recording the time and volume of each sample. **Results:** The assay permits analysis of the sunscreen agents in biological fluids, including bovine serum albumin

(BSA) solution, human plasma and skin strips. The assay was linear ($r^2 > 0.99$) with minimum detectable limits of 0.8 ng for oxybenzone, 0.3 ng for octylmethoxycinnamate, and 2 ng for homosalate and octylsalicylate. The inter- and intra-day variation for the four sunscreens was less than 3% at the upper end of the linear range and less than 6% at the lower end. Recoveries of sunscreens from plasma and 4% BSA solution were within the range 91-104%. Higher amounts of sunscreen agents were recovered from the upper layers of stratum corneum at 30 minutes. At 4 and 8 hours post application, similar depth of penetration profiles were obtained but with overall lower sunscreen concentration. A significant amount of oxybenzone was measured in the plasma and urine. Upto approximately 1% of oxybenzone and its metabolites were detected in the urine. **Conclusions**: The HPLC assay developed is sensitive, simple, rapid, accurate and reproducible. Results from the preliminary study demonstrate significant penetration of sunscreen agents into and across the skin. **Keywords**: HPLC Sunscreen Penetration Human epidermis Systemic absorption

37. IMPARTING A BONE MINERAL AFFINITY ONTO GLYCOPROTEINS THROUGH THE CONJUGATION OF BISPHOSPHONATES.

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Purpose: To develop a novel means of conjugating bisphosphonates onto the carbohydrate moieties of glycoproteins to enhance bone affinity. **Methods:** Chemistry using succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC): Based on an optimized procedure for albumin, bovine fetuin lysine groups were reacted with the heterobifunctional crosslinker SMCC to introduce thiol-reactive maleimide groups onto the glycoprotein. The product was then reacted with aminobisphosphonate (aminoBP), whose $-NH_2$ had previously been converted to –SH using 2-iminothiolane, to yield a glycoprotein with bisphosphonates conjugated onto its protein core. Chemistry using 4-(maleimidomethyl)cyclohexane-1-carboxyl-hydrazide (MMCCH): After oxidizing the carbohydrate groups of fetuin using sodium periodate, the hydrazide group of the heterobifunctional crosslinker MMCCH was reacted with the newly introduced aldehydes to yield a maleimide-containing glycoprotein. Thiolated aminoBP was then reacted with the reaction product to yield a glycoprotein with bisphosphonates conjugated onto its carbohydrate moieties. Analysis of the Conjugates: Using the Bradford and an organic phosphate assays for protein and aminoBP concentrations in solution, respectively, conjugation efficiency was determined for each chemistry. Conjugate affinity for synthetic hydroxyapatite and various other bone matrices were assessed in vitro. **Results:** Using either chemistry, increasing reactive reagent concentrations led to a proportional increase in the number of bisphosphonates conjugated onto the fetuin (up to 7.0 and 16.0 aminoBPs/fetuin, for MMCCH and SMCC respectively). Although this increase in the number of conjugated aminoBPs corresponded to a proportional increase in the conjugates' affinity for various bone matrices, the MMCCH-conjugates gave a 2.6-, 2.0-, 30.5-, and 1.84-fold increased affinity for untreated, ashed, demineralized bone and hydroxyapatite, respectively, as compared to the SMCC-conjugates. Both conjugates exhibited a pH-independent, equally slow degradation in adult bovine serum containing media. **Conclusions:** Our results suggest that the conjugation of bisphosphonates either onto fetuin's protein core or onto its carbohydrate groups (using the SMCC and MMCCH chemistries, respectively) increases the glycoprotein's affinity for HA.

38. PHYSICOCHEMICAL PROPERTIES OF NEUROMUSCULAR BLOCKING AGENTS AND THEIR IMPACT ON THE PHARMACOKINETIC-PHARMACODYNAMIC RELATION

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Purpose: Among the factors influencing the onset of action of neuromuscular blocking agents (NMBA), the potency (EC_{50}) and the rate of equilibration between blood and the effect compartment (K_{e0}) have been highlighted by many. Although these parameters are intrinsically influenced by the drug physicochemical characteristics, the impact of lipid solubility, molecular weight and protein binding on pharmacokinetic-pharmacodynamic (PK-PD) parameters has not been established for most NMBA. **Methods:** The octanol/phosphate buffer partition coefficients (log D) of various NMBA (ORG9991, vecuronium, rocuronium, mivacurium isomers (*cis-cis*, *cis-trans* and *trans-trans*), doxacurium, pipecuronium, cisatracurium, atracuronium, succinylcholine) were determined. The degree of protein binding for each drug was measured using an ultrafiltration technique. PK-PD parameters were obtained from selected clinical studies. Correlations between physicochemical parameters (including molecular weight, MW) and PK-PD parameters were assessed by linear or multiple linear regressions. **Results:** A wide range of logD (-4,15 for succinylcholine to 0,25 for ORG 9991) and protein binding (fu from 18 % for ORG9991 to 80 % for succinylcholine) is observed for NMBA. MW combined with either lipid solubility (r^2 = 0,91; p = 0,009) or protein binding (r^2 = 0,86; p = 0,018) were highly correlated with potency while for k_{e0} a greater degree of correlation was ob-

tained when both lipid solubility and protein binding (r^2 = 0,94; p= 0,004) were included. **Conclusion:** Physicochemical properties ought to be taken into account when designing new NMBA.

39. INTERSTITIAL MUSCLE CONCENTRATIONS OF ROCURONIUM UNDER STEADY-STATE CONDITIONS IN ANESTHETIZED DOGS: ACTUAL VERSUS PREDICTED VALUES.

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Purpose: To compare the effect compartment concentration and peripheral concentrations of rocuronium derived from pharmacokinetic-pharmacodynamic (PK-PD) modeling to the actual concentration sampled at the site of action (biophase) by microdialysis under steady-state conditions. **Methods**: Anesthesia was induced and maintained with pentobarbital in eight dogs (7-20 kg). Arterial and venous catheters as well as microdialysis probes were inserted followed by *in vivo* calibration. Force of muscle contraction was measured at the forelimb. Each dog received a 2-min rocuronium infusion of 0.3 mg/kg followed by a 120 min iv infusion of 60 µg/kg/min to reach steady-state conditions. Muscle interstitial fluid (ISF) was collected by microdialysis every 40 minutes for 120 minutes. At the end of the infusion, arterial samples were collected each 2 minutes for the 10 first minutes and at 20 minutes intervals for the remaining 120 minutes. Protein binding was determined *in vitro*. After solid-phase extraction, rocuronium concentration was determined in biological samples by an HPLC method coupled to EC detection. Concentrations in the peripheral (C_2) and effect (C_e) compartment at various times were derived using standard parametric PK-PD models and presented as the unbound values (mean ± S.D). **Results:** Under steady-state conditions, no statistical difference was observed between measured muscle interstitial concentrations ($C_{ISF,\ u}$ = 1353 ± 265 ng/ml) and their mathematical estimates of peripheral ($C_{2,u}$ = 1523 ± 575 ng/ml) or effect compartment concentrations ($C_{e,u}$ = 1662 ± 738 ng/ml). **Conclusion:** Quantitative *in vivo* measurement of the interstitial fluid concentration of rocuronium at the effect site (muscle tissue) confirms the basic assumption made in the PK-PD link model i.e. the free concentrations of the effect and central compartments are equal under steady-state conditions. For muscle relaxants, the peripheral concentration derived with the two-compartment model may prove to be an useful asset to gain insight into physio-anatomical compartments.

40. REMIFENTANIL PERIPHERAL ELIMINATION IN MUSCLE AND BRAIN OF ANESTHETIZED DOGS UNDER STEADY STATE CONDITIONS.

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Purpose. To measure peripheral elimination of remifentanil (RMF) in dogs under steady state conditions by simultaneous sampling in the central (blood) and peripheral (muscle) compartment. To calculate RMF extraction ratios (ER) for muscle and brain (effect compartment). **Methods**. Mongrel dogs (n=7, 8.8-20 kg) were anesthetized with pentobarbital (30 mg/kg). The femoral artery was cannulated and blood drawn for protein binding determination. Catheters were inserted in the jugular and femoral veins for blood sampling and respective arterio-venous gradient. A spinal needle was introduced in the cisterna magna for cerebrospinal fluid (CSF) puncture. Microdialysis probes were inserted in the muscle of the hindlimb and calibrated *in vivo*. A 60 min infusion via the femoral vein was started. Thirty minutes dialysate fractions were collected ($ISF_{u,ss}$). CSF and arterio-venous blood samples were drawn simultaneously at 30, 40 and 50 min. **Results**. Unbound fraction value was used to correct the total plasma concentrations. Mean RMF protein binding was 71 ± 8 %. Mean $ISF_{u,ss}$ concentration of 311 ng/mL represented 69 ± 13 and 110 ± 14 % of that of the arterial and venous blood, respectively. Similarly, mean CSF concentration of 236 ng/mL represented 53 ± 6 and 82 ± 18 % of the arterial and venous free concentration, respectively. The mean ER were 36 ± 7 % for muscle and 31 ± 12 % for brain. **Conclusion**. The results confirmed a substantial extraction of RMF by muscle and brain and proved that muscle $ISF_{u,ss}$ and CSF_{ss} concentrations are lower than central concentrations (Cp_{ss}). This evidence should foster the development of new PK models for drugs undergoing peripheral elimination. RMF venous concentrations would be more representative of interstitial tissue concentrations under steady state conditions. This has definite implications, as drug concentrations at the site of action are more relevant for the concentration-effect relation.

41. FEASIBILITY OF ORAL CASSETTE DOSING IN HIGH-THROUGHPUT PHARMACOKINETIC *IN VIVO* SCREENING.

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Purpose. In Drug Discovery, intravenous cassette dosing (coadministration of several compounds) is used as a high-throughput, resource-saving approach to obtain preliminary information on the *in vivo* pharmacokinetic profile of new chemical entities (NCEs). Due to concerns of increased risk of drug-drug interactions, oral cassette dosing has not been used extensively. We evaluated the feasibility of oral cassette dosing

as a first *in vivo* screen of oral bioavailability. **Methods.** Following oral pharmacokinetic studies carried out using single drug administrations (5 μmol/kg), five NCEs (1-5), selected to represent "low" to "high" bioavailability, were coadministered (total dose 24 μmol/kg) by oral gavage to male rats. Plasma levels were measured by HPLC/MS. For each NCE, pharmacokinetic parameters were derived non-compartmentally and compared following single drug *vs*. cassette dosing. **Results.** Pharmacokinetic parameters were not derived for NCE 5 due to too low plasma concentrations, following both single drug and cassette dosing. For NCEs 1-4, key parameters determined from both experimental paradigms were comparable. The results, expressed as cassette/single ratios, averaged 0.83 for $AUC_{0\text{-inf}}$ and 1.12 for Cmax. More importantly, bioavailability ratios approximated 0.82. For NCE 2, bioavailability was ≤10% in both types of experiment, whereas for the other 3 NCEs, it ranged between 27 and 63%. **Conclusion.** Based on the oral cassette pharmacokinetic study, NCE 2 and 5 would have been discontinued because of insufficient exposure/bioavailability, while compounds 1, 3 and 4 would have been progressed to further testing. Single drug dosing studies would have led to the same decisions. This favourable outcome will be confirmed with further experiments.

42. JOINT ASSAY OF DESETHYLAMIODARONE AND AMIODARONE IN RAT PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Purpose: Amiodarone (AM) is an important antiarrhythmic drug. We previously reported an assay for AM, and recently received a gift of desethylamiodarone (DEA), its active metabolite, from Wyeth Research (Monmouth Junction, NJ, USA). The purpose of this work was to confirm and validate the ability of the previous HPLC method for AM to jointly assay DEA in rat plasma. **Methods:** All calibration and validation samples were based on 0.1 mL of rat plasma. To the plasma was added 0.03 mL of (±)-ethopropazine HCL (0.1 mg/mL) as internal standard (IS) and 0.3 mL acetonitrile. Tubes were vortex-mixed (5 sec) and centrifuged (2 min) at 3000 *g*. The supernatant was transferred to clean tubes, and 0.3 mL of phosphate buffer (pH 5.9) and hexane (3 mL) were added. After vortexing (30 sec) and centrifuging (3 min), the upper layer was transferred to clean tubes and dried *in vacuo*. Residues were reconstituted in mobile phase (0.15 mL) and 30-60 mL injected into the HPLC. Mobile phase consisted of methanol: [25 mM potassium phosphate-3mM sulfuric acid-3.6 mM triethylamine]:acetonitrile (63:25:12.5), pumped at 1.5 mL/min. A Waters RCM C_8 analytical column was used for separation. The initial UV detection wavelength was 254 nm, then switched to 242 nm at 7 min. **Results:** The IS, AM and DEA eluted at 3.5, 11 and 13.5 min, respectively. Weighted (1/conc) standard curves based on analyte:IS height ratios were linear over the range of 35-1000 ng/mL (r^2>0.99). The intraday average within-run precision and average accuracy for DEA ranged from 4.21 to 12.9% and from 91.4 to 110.3%, respectively. **Conclusions:** The assay was reproducible and met established criteria for validity. The lower limit of quantitation of DEA was 35 ng/mL based on 0.1 mL of rat plasma, the same as previously reported for AM in rat plasma.

43. A KINETIC MODEL FOR THE TIME COURSE OF ETHANOL IN PLASMA AND AMNIOTIC FLUID OF PREGNANT WOMEN.

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Purpose: To develop a PK model to simultaneously describe the kinetics of ethanol in maternal blood and amniotic fluid of pregnant women. **Methods:** Ethanol concentrations simultaneously measured in the maternal blood and in the amniotic fluid of six pregnant women were obtained from the study of Brien *et* al. (1983). Ethanol 300 mg/kg was orally administered and the ethanol and acetaldehyde concentrations were measured in the maternal venous blood and amniotic fluid by GLC. Data of the time-course of ethanol in every patient were published as figures which were scanned and digitalized. For maternal blood concentrations, the equation was:

$$\frac{dC_{EtOH-P}}{dt} = -\left(k_{12} + \frac{Vm_{EtOH}}{Km_{EtOH} + C_{EtOH-P}}\right) \times C_{EtOH-M} + \left(k_{21} \times C_{EtOH-AF}\right)$$

The kinetics of ethanol in amniotic fluid was modeled by using the following equation:

$$\frac{dC_{EtOH-AF}}{dt} = k_1 \times C_{EtOH-P} - k_2 \times C_{EtOH-AF}$$

Results: The t_{max} in AF was longer than in maternal blood (p=0.001, unpaired Student t test). The mean C_{max} in AF was 60% lower than the C_{max} reached in maternal blood (p=0.036, signed rank test). However, the $AUC_{0\text{-}3.5\ h}$ in AF was only 16% lower than plasma (p=0.059, signed rank test). The kinetic model successfully fitted to the ethanol concentrations in both maternal blood and amniotic fluid. Slow k_1 produced a slow output of ethanol from the amniotic fluid, and inversely high values of k_1 favored a fast output of ethanol from the amniotic fluid. The k_2 resembled an absorption rate; small values produced a delay in the time to reach the t_{max} of ethanol in the amniotic fluid. **Conclusion:** We provided a model to explain the kinetics of ethanol in plasma and amniotic fluid of pregnant women. This kinetic model can help to understand the complex interrelationship between mother and fetus in the

context of interpatient variability leading to fetal alcohol syndrome. **Acknowledgements:** The study was supported by the Hospital Infantil de México and the Sistema Nacional de Investigadores (AANO), and by the CIHR (GK). **References:** Brien JF, et al. Disposition of ethanol in human maternal venous blood and amniotic fluid. Am J Obstet Gynecol 1983; 146: 181-6; Fujimiya T, et al. Alcohol Clin Exp Res 2002; 26: 49S-54S.

44. THE STUDY OF ADHESIVE NANOPARTICULAR VECTOR TAILORED FOR THE TREATMENT OF CANCER BY INHIBITION OF ANGIOGENESIS

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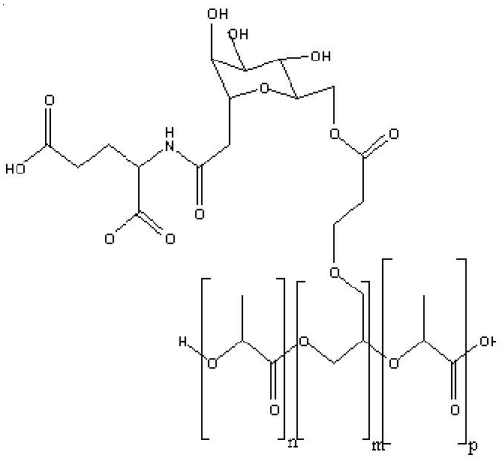
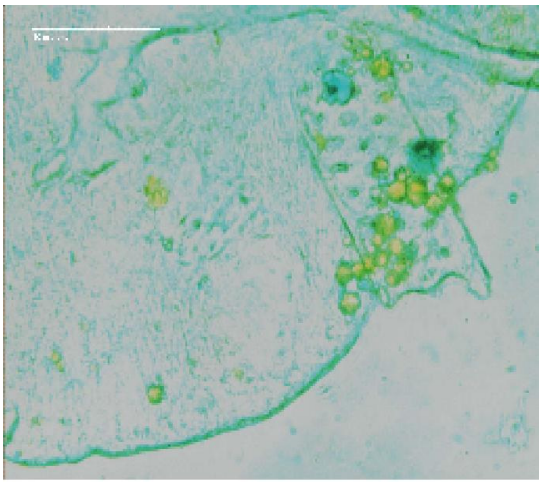
Purpose: Conventional weapons for cancer treatment are not specific. A now popular idea is to cut off the tumour feeding by inhibition of angiogenesis. The method used is to encapsulate anti-angiogenic drugs into microspheres made of a biodegradable polymer on which is grafted a selective ligand for Selectin-E. Those Selectin-E are over expressed on vascular vessels during angiogenesis and represent the area of interest. **Methods:** A polymer made of functionalized polylactide (PLA) on which is grafted the ligand at 1% has been synthesized (Figure 1). Microspheres are assembled with this polymer by single emulsion/evaporation method. To visualize the microspheres, they were labelled by an orange or blue dye. Mean diameter of microspheres have been measured by image analysis using Zeiss optical microscope mounted with a CDD digital camera. Ex vivo experiments were realised by using those microspheres with mesenteric rat vessels. Rats where treated by L-NAME for 3 weeks to induce inflammatory response. **Results:** The results prove that the active targeted microspheres (in orange) stick more to the vascular endothelium in comparison with microspheres used for control (in blue). Those microspheres (in blue) made of polymers without ligand were washed out during the process and did not adhere (Figure 2).





Furthermore, small microspheres seem to have a better adhesion than bigger ones. A saturation threshold of the vascular endothelium has been finally found by using different concentration of microspheres in suspension. It has been shown that a saturation plateau occurs at 0.18 mg/ml. The determination of maximum selectin site number in a defined area has been evaluated at 1.8 for 1000 square-micrometers. **Conclusion**: Consequently, the bioadhesive drug carrier we synthesized can specifically target Selectin E at the endothelial surface and could be of interest in the treatment of several pathologies. **Reference :**G. Leclair, dissertation thesis, U. Montreal, 2003, pp182. **Acknowledgement**: We are grateful to the National Summer Student Research Program funded by the Merck Company Foundation for their support.

45. SECOND DERIVATIVE SPECTROPHOTOMETRY FOR SIMULTANEOUS DETERMINATION OF DNA AND ALL-TRANS RETINOIC ACID IN PLA NANOSPHERES.

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Purpose: Combined angiosuppressive therapy has shown to be more effective than the corresponding monotherapy in the aim of treating tumor malignancies [(1)]. The microencapsulation of two antiangiogenic agents in biodegradable nanospheres prepared by the double emulsion solvent evaporation method is achieved using one hydrophilic agent, a model DNA (I), and another lipophilic drug namely, all-trans retinoic acid (II). A method is described for the simultaneous assessment of both molecules by solving the second derivative spectra (^{2}D) for the two components using Vierordot's method [(2)]. **Methods**: The Accuracy of the modified ^{2}D method was checked by analysing

six synthetic mixtures of I and II at various concentrations within their respective linearity ranges, at concentration ratios (I: II) ranging from 1:0.0012 to 1:0.03. Nanospheres were prepared by w/o/w emulsion solvent evaporation method, where 2.5 mg of I was dissolved in 0.5 ml of the internal aqueous phase and 10 mg of II, was incorporated with the organic polymeric phase. **Results**: The interference was greatly reduced by the application of the derivative function (Figures 1a and 1b). By solving the obtained spectra with Vierordot's method, the obtained recovery values ranged from 98.5% to 101.2% and from 99.6% to 100.8% for II, and I respectively. Furthermore, the validity of the technique for determination of extracted ingredients from prepared nanospheres was confirmed by standard addition method for the two components. **Conclusion**: In addition to the sensitivity and the reproducibility, the modified 2D method is shown to be simple and fast. Further adaptation of the technique is possible for the release studies of both components from nanospheres. References: (1) Bergers G, Javaherian K, Lo KM, *et al.*; Science (1999), 284: 808-812. (2) M.A. Korany, A.M. Wahby, M.A. Elsayed and S. Mandour; Anal. Lett. (1984), 17 (B12):1343-1446.

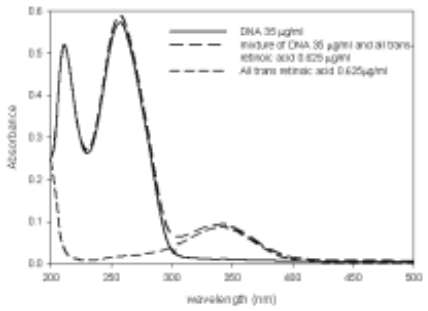

Figure 1a. Absorbance spectra of DNA 35 µg/ml, Trans retinoic acid 0.625 µg/ml, and their mixture in tris-EDTA buffer pH 8

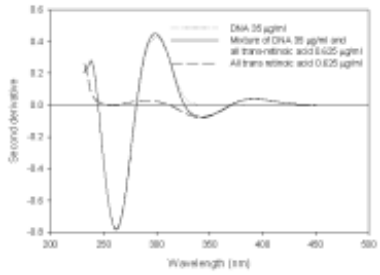

Figure 1b. Second derivative spectra of 35 µg/ml DNA, 0.625 µg/ml trans retinoic acid, and their mixture in tris- EDTA buffer pH 8

46. SYNTHESIS AND CHARACTERIZATION OF A NOVEL CLASS OF GRAFTED AND CROSS-LINKED BIOPOLYMERS.

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Introduction. The aim of this work is to synthesize versatile branched polyester allowing the attachment of the pendant group by a single step reaction to yield a biodegradable polymer used for the delivery of DNA. The conception of a versatile function that allows the grafting of a variety of labels has been chose. Polyesters such as PLA are biomaterials widely studied because of their good properties. Polymers having a pendant group could be grafted with various molecules such as other polymers, lipids, ionisable function or antibodies. **Experimental method.** (cis)-Dilactide and allyl glycidyl ether were added in a round bottom flask with tetraphenyltin as catalyst. The mixture has been heated at 180 ˆC for 6 hours. The resulting polymer was dissolved in chloroform and was purified by precipitation in water. The double bond was oxidized to hydroxyl group by hydroboration and the carboxylic group was obtained by oxidation using the Jones mixture (fig 1). The ligand has been synthesized in several steps (fig 2) and was linked to polymer by esterification.

Figure 1 Scheme of polymer synthesis.

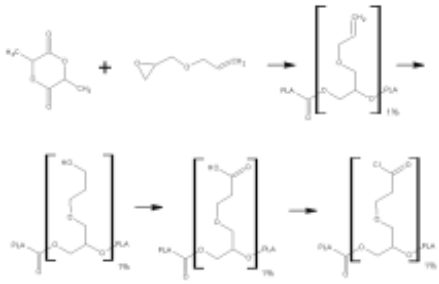
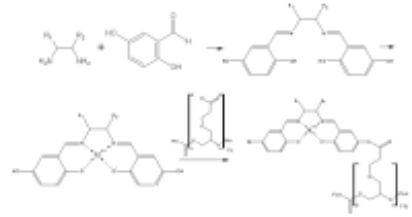


Figure 2 Scheme of pendant group grafting.



Polymers have been characterized by GPC and DSC. The ligands with Zn metal are not charged and were used to prove by RMN the structure of the complexe. RMN, IR and elemental analysis presented structural proof. **Results.** The global yield of polymer synthesis was about 75% before the grafting step. The ligand synthesis global yield was about 50%. Table 1 and 2 show respectively the tg obtained by DSC and molecular weights (polydispersity) by GPC. The values are similar for all kinds of polyesters. The charged cross-linked polymers obtained

by esterification of polymer and ligand (fig 1-2) gave possibility of treating cancer by complexation of DNA. **Conclusion.** A new class of polyesters with different pendant groups can be used for pharmaceutical and for biomedical application.

Table 1: Tg of PLA+ligand.

metal	R= cyclohexane	R= ethylene	R1 = R2 = phenyl
Mn^{2+}	159°C	154°C	169°C
Cu^{2+}	171°C	174°C	159°C
Zn	153°C	164°C	165°C

Table 2 : Molecular weights and polydispersity of PLA+ligand.

metal	R= cyclohexane	R= ethylene	R1 = R2 = phenyl
Mn^{2+}	Mn 5549 Mw10569 I 1.905	Mn 2720 Mw 4088 I 1.503	Mn 2420 Mw 3403 I 1.406
Cu^{2+}	Mn 5512 Mw 14397 I 2.612	Mn 2123 Mw 2569 I 1.210	Mn 2204 Mw 2757 I 1.251
Zn	Mn 5541 Mw 13327 I 2.405	Mn 3154 Mw 4980 I 1.579	Mn 2792 Mw 5157 I 1.847

47. DNA-CONTAINING NANOSPHERES MADE OF BIODEGRADABLE POLYMERS: OPTIMIZATION OF PREPARATION CONDITIONS AND PHYSICAL CHARACTERIZATION

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Purpose. To prepare and characterize biodegradable nanospheres containing a model DNA, under variable preparation conditions. **Methods.** Nanospheres were prepared according to a multiple emulsion solvent evaporation technique. Polymers used were PLGA, PLA, PLA-PEG triblock and multiblock co-polymers. Homogenization pressure was varied from 5,000 to 20,000 psi. Concentration of PVA as emulsifying agent was also changed from 0.1% to 1%. Sorbitol used as a cryoprotectant was added at various concentrations ranging from 0.5% to 5%. Nanospheres were characterized for their particle size by photon correlation spectroscopy. Surface area and porosity were measured by gas adsorption. Shape and surface were characterized by scanning electron microscopy and atomic force microscopy. Encapsulation efficiency and release kinetics of DNA were evaluated. **Results.** Nanospheres with appropriate size (<1µm) were obtained (Figure1). Particle size did not depend on the polymer used, but rather the preparation conditions. Increasing homogenization pressure decreased nanosphere particle size up to a certain limit. Particle size was inversely proportional to PVA concentration in the external aqueous phase. Sorbitol prevented particle aggregation and resulted in smaller particle size than the control nanospheres. Homogenizing pressure, PVA and sorbitol concentration influenced porosity in terms of total pore volume and pore volume distribution. Polymer type had an influence on the total porosity, pore size distribution, as well as the release rate of DNA. A maximum encapsulation efficiency of 70% was achieved. DNA release started with a burst occurring within the first hours, followed by a slow release phase over ten days. **Conclusion.** Results show that the double emulsion method described in this work was efficient to prepare nanospheres of appropriate size, and ensuring a controlled release of DNA. Most suitable formulation parameters will be selected for routine batch preparation. *This work has been presented in the AAPS Annual Meeting & Exposition, November 2002, Toronto, Ontario, Canada.*

48. PREPARATION OF HOLLOW PARTICLES FOR CELL ENCAPSULATION BY A METHOD OF POLYESTER CORE DEGRADATION.

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Purpose: Implantation of encapsulated cells in hydrogel particles of less than 1 mm (micro-encapsulation) has been proposed as a cell synthesized biomolecule delivery system. Encapsulation provides immuno-isolation, protecting foreign cells from host immune system while nutriments, oxygen and therapeutic products freely diffuse through capsule walls. Hydrogel hollow particles can be used in many applications as drug reservoir, nanoreactor and drug carrier. **Methods**: We designed a new method to produce the desired hydrogel particles. Hydrogel microcapsules were prepared by a three-step original procedure: first, synthesis of a core particle, followed by coating with a layer of epichlorohydrin cross-linked amylopectin gel, and finally selective chemical degradation of the core particle. Hollow particles were characterized for their size and morphology by light microscopy and for their permeation by diffusion of FITC-dextrans. Optimizations were carried out to improve the hollow particle yield having appropriate size. **Results**: Initial experiments used amylo-pectin cross-linked with trimetaphosphate as core particle material. However, selective chemical degradation was difficult to achieve. In further assays, polyesters were used successfully for the preparation of core particles. Polyesters (i.e. Polylactide) are degraded in basic conditions, while epichlorohydrin cross-linked amylo-pectin gels are stable for an appreciable incubation time. The mean size of the hollow particles is about 500 µm and the morphology is very spherical with one cavity about 300µm diameter. The permeability study conducted with FITC-Dextrans shows molecular weight cut-off between 10KD and 70KD. **Conclusion:** These preliminary results show that the new method has the

potential to become a standard procedure to obtain hydrogel hollow particles. Although the method has been designed to produce capsules for cell encapsulation, it can be extended to the preparation of hollow particles for application such as drug delivery, cosmetic, and biotechnology.

49. HPLC DETERMINATION OF DICLOFENAC IN HEALTHY VOLUNTEERS, AFTER A SINGLE DOSE ADMINISTRATION.

***Louis Abreu,*[1], Santana, D.P.[2], NUDFAC – Núcleo de Desenvolvimento Farmacêutico e Cosmético, Universidade Federal de Pernambuco, Pernambuco, Brasil**

Absent

50. CHARACTERIZATION OF A NOVEL METABOLIC PATHWAY OF FLUOXETINE: N-HYDROXYLATION

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Purpose and objective : The only metabolic pathway known in the disposition of the secondary amime fluoxetine is N-demethylation to norfluoxetine which represents about 10 % of the administered dose in human; the remaining of the dose in non accounted for except for about 5 to 10 % of the dose which is excreted as unchanged fluoxetine in the urine. The hypothesis to be tested in this study was that fluoxetine is metabolized by N-hydroxylation and if so, to characterize the metabolic properties of the formation of the N- hydroxylated derivative in comparison to those of the known N-demethylation pathway using hepatic microsomes. **Methods:** N-Hydroxyfluoxetine was synthetized by reacting fluoxetine with m-chloroperbenzoic acid according to an established procedure. A reverse-phase hplc assay was developed for the determination of fluoxetine, its N-hydroxylated derivative and norfluoxetine. Routine determinations of the N-hydroxylation and N-demethylation pathways in microsomal incubations were carried out with specific spectrophotometric assays. Fluoxetine was incubated with hepatic microsomal preparations obtained from rats (Sprague-Dawley and Dark Agouti), Syrian hamsters, New Zealand white rabbits and human (male) in the presence of a NADPH-generating system at pH 7, 4 and 37° C under aerobic conditions. **Results:** N-Hydroxyfluoxetine was identified by liquid (hplc) and thin-layer chromatography in the neutral organic extracts following incubation of fluoxetine with liver microsomes. The rate of formation of the N-hydroxylated metabolite was dependent on the microsomal protein and fluoxetine concentrations and of the time of incubation. This metabolite was unstable in solution ($t_{1/2} \cong 60$ min) and the addition of 0,5 mMglutathione stabilized the compound and permits reliable determinations. Both metabolites, norfluoxetine and the N-hydroxyfluoxetine, were formed in all species including human. In addition, all species expressed greater activities (10 to 30 times) for the N-hydroxylation. The rate of N•demethylation in microsomes from Sprague-Dawley rats was 3 times that obtained in incubations containing microsomes from Dark Agouti rats indicating the involvement of CYP2D in this pathway. No such variation was obtained for the N-hydroxylation of fluoxetine between both strains of rats. On the other hand, concentration-dependant inhibition of the rate of the formation of the N-hydroxylated metabolite by cimetedine and gender variations indicates the involvement of CYP2C in the N-hydroxylation pathway. **Conclusion:** N-Hydroxyfluoxetine was identified as a metabolite of fluoxetine following incubations with hepatic microsomes. In vitro studies indicate that the rate of N-hydroxylation is much higher than that of the known N-demethylation pathway. A knew metabolic pathway in the disposition of fluoxetine has been described. Funding from CIHR.

51. THE POLYMORPHISMS OF GENES ENCODING ENZYMES TARGETED BY METHOTREXATE AND THE OUTCOME OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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PURPOSE Thymidylate synthase (TS) catalyzes the conversion of dUMP to dTMP. It is an essential enzyme in proliferating cells and an important target for chemotherapeutics including antifolate methotrexate (MTX), a key-component of ALL treatment. We recently reported that a 3R variant of TS promoter polymorphism might influence ALL outcome. Here we analyzed several additional variants in MTX action pathways. These include a 6-bp variation in the 3'UTR of TS gene that might affect mRNA TS stability, as well as an A870G polymorphism in the cyclin D1 (CCND1) gene leading to different splicing forms that may differentially regulate MTX targets. The syntheses of specific folate cofactors required for a folate cycle enzyme function are mediated by methylenetetrahydrofolate reductase (MTHFR) and methylene tetrahydrofolate dehydrogenase (MTHFD). Two polymorphisms in MTHFR gene, C677T and A1298C, leading to reduced enzymatic activity, and G1958A in MTHFD gene leading to amino-acid substitution, were included in the analysis. **METHODS** Using PCR-based assays we have analyzed TS, MTHFR, MTHFD and CCND1 polymorphisms in 207 French-Canadian children with ALL who were treated with 3 consecutive DFCI Consortium protocols. The impact of these polymorphisms on ALL outcome was analyzed by EFS estimates for the patients with and without indicated variants. **RESULTS** Cox regression analysis with the inclusion of the relevant clinical prognostic factors showed that

individuals who were CCND1 A homozygous had worse prognosis than CCND1 G carriers (HR=2.8, 95%, CI= 1.3-6.0, p=0.01). This impact was particularly apparent in individuals with the TS 3R variant (p<0.0001). The patients with the MTHFR T677A1298 haplotype tended to be at higher risk of event compared to C677A1298 individuals (HR=7.5, 1.0-58.2, p=0.05), whereas the 6-bp TS variant, MTHFD G1958A substitution, or MTHFR C677C1298 haplotype did not seem to influence ALL relapse rate. **CONCLUSION** Pharmacogenetic screening might have important consequences for clinical management of ALL patients. This work is supported by the Fonds de la Sante en Recherché du Quebec, Canadian Institute of Health Research, Leukemia Research Fund of Canada and Research Center of Ste-Justine Hospital.

52. BEYOND THE FRACTAL CHARACTERIZATION OF POROUS MEDIA: THE MODIFIED AUTO-CORRELATION METHOD

***Fahima Nekka* and Jun Li**

Purpose: In porous media, it is well known that microstructure fluctuations can have important consequences on bulk mechanical and rheological properties. These physical structures can share the same fractal dimension in spite of their different appearance. The most popularized concept in fractal analysis of structure was the fractal dimension. Only in the last decade, additional tools to get rid of the degeneracy character of this parameter have been developed. The few ones devoted to texture have proved to be still degenerate in rather simple cases. In order to characterize the fine details of a structure, mainly represented by the distribution and shapes of its gaps, we developed a new formalism that provides a complete characterization of the geometrical organization of porous media. **Methods**: The method developed is based on a modification of the auto-correlation method, which is widely used in engineering. It can be viewed as a two-point joint moment (autocovariance) of the structure indicator function. This explains in a way why the measure given by our method completes naturally the information obtained from pointwise descriptors. **Results**: The method has been tested on known sets as well as on porous models. It has resulted in a complete differentiation between structures having the same fractal dimension. **Conclusion**: This method offers a more precise description of the fine texture of porous structure generally undistinguishable by existing methods. The results of the examples we studied are promising for a wider use.

53. POLYCAPROLACTONE-*BLOCK*-POLY(ETHYLENE OXIDE) MICELLAR DELIVERY SYSTEM FOR SEX STEROIDS

***Patrick Lim Soo*[1], Patricia Davidson[1], Dusica Maysinger[2] and Adi Eisenberg[1], [1]Department of Chemistry, McGill University, Montreal, Quebec, Canada, [2]Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada**

Purpose. Hormone imbalance leads to the impairment of the reproductive, cardiovascular and the nervous systems. We propose polycaprolactone-*block*-polyethylene oxide (PCL-*b*-PEO) micelles as a versatile and efficient nano-delivery vehicle for the sex steroids, 17b-estradiol and testosterone. **Methods.** The extent of incorporation and release were evaluated using fluorescence spectroscopy and Enzyme-Linked Immunosorbent Assay (ELISA) methods. Dynamic light scattering, transmission electron microscopy and differential scanning calorimetry were also used to evaluate the effectiveness of PCL-*b*-PEO micelles as delivery vehicles. **Results.** The maximum loading efficiency of 17b-estradiol is 97% in contrast to that of testosterone, which reaches a maximum of 30%. The compatibility between the sex steroids and the polycaprolactone core is favourable as evident from the high loading efficiencies. The release of estradiol was biphasic with an initial small burst followed by a sustained release. The data were fitted to the Higuchi model and release was shown to be diffusional. **Conclusion.** PCL-*b*-PEO micelles show great promise as a delivery vehicle for the sex steroids, 17b-estradiol and testosterone. Poster originally presented at 226th American Chemical Society meeting in New Orleans (March 23-27, 2003).

54. BIODEGRADABLE CIPROFLOXACIN-LOADED CONTRAMID® IMPLANTS AS A LOCAL THERAPY OF OSTEOMYELITIS IN DOGS

***C. Désévaux**, B. Lussier, V. Lenaerts and P. Dubreuil, *Labopharm Inc., Laval, Quebec, Canada**

Purpose. As an excellent biocompatibility has been reported for cross-linked high amylose starch (Contramid®), the purpose of this study was to demonstrate the efficacy of Contramid® implants as a sustained antibacterial delivery system for the treatment of induced osteomyelitis. **Methods.** Contramid® implants (7-mm diameter; 200 mg) containing 40 mg ciprofloxacin (CFX) were prepared by direct compression. Six adult Beagle dogs weighing 10 to 13 kg were used. The lateral side of the left femur was exposed. As a foreign body, a cis-cortical and a trans-cortical screw were inserted in the proximal and the distal diaphysis part, respectively. Moreover a cortical window was cut around the trans-cortical screw to create a bone sequester. A suspension of the *S. aureus* strain ATCC29213 was instilled into both sites. Four weeks (W4) after inoculation, debridement of infected sites was performed, screws were withdrawn and eight Contramid® implants (320 mg CFX) were positioned along the femoral diaphysis. Throughout the study period (W0 to W10), animals were monitored with clinical exams and radiographs. Bacterial evaluation was performed from collected bone at W4 and W10. **Results.** Some

dogs developed a slight fever and/or an edema of the operated leg after inoculation. Lameness was recorded from W0 to W4. An osteomyelitis was successfully established at W4. Lesions were observed on radiographs and at macroscopic examination and bacterial counts were highly positive. Treatment of osteomyelitis with Contramid® implants was efficient (W4 to W10). Lameness and radiographic lesions decreased over time. At W10, no evidence of osteomyelitis was noted at macroscopic examination. Bacterial counts were negative and dramatically decreased in four and two animals, respectively. **Conclusion.** In this study, we demonstrated for the first time the successful efficacy of Contramid® implants loaded with CFX as a sustained delivery system for local antibacterial therapy of osteomyelitis. This work has been presented at the 29th International Symposium on Controlled Release of Bioactive Materials (Controlled Release Society), July 2002, Seoul, Korea; Proceed. Int'l Symp. Control. Rel. Bioact. Mater., 29 (2002) pp 1167-1168

55. EVALUATION OF SOLID DISPERSIONS ON PREDNISONE DISSOLUTION

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Absent

56. AN INVESTIGATION INTO THE PHASE MISCIBILITY OF IBUPROFEN-LOADED HPMC FILMS USING MTDSC, PULSED FORCE ATOMIC FORCE MICROSCOPY AND SEM

Samana Hussain and *Duncan Craig*, School of Pharmacy, Queen's University of Belfast

Purpose: There is a continuing interest in the use of drug-loaded polymers as controlled release devices, implants and medical devices. However, the miscibility of drugs within the polymeric matrices is often poorly understood. In this investigation, we describe the combined use of thermal analysis and microscopic methods as a means of both imaging regions of phase separation and to ascertain the degree of miscibility of the components of ibuprofen-loaded HPMC films. **Methodology**: Films were prepared by dissolving the two materials in ethanol-water mixes (2:3), followed by casting and storage over phosphorous pentoxide. Thermal studies were conducted using a T_{zero} Modulated Temperature DSC (Q1000, TA Instruments) using indium, tin, n-octadecane and aluminium oxide as calibrants. Complementary thermogravimetric analysis studies were performed using a Hi-Res TGA 2950 (TA Instruments). Pulsed force atomic force microscopy was performed using a TM Microscopes Explorer AFM, using 1995-00 single cantilever probes with a nominal force of $0.2Nm^{-1}$. SEM was performed using a JOEL 6400 scanning microscope. **Results**: T_{zero} scans of the individual components and the loaded films indicated that the glass transition of the HPMC (measured as 161°C for the single component system) was reduced to 139°C and 132°C for the 6% and 10% drug loaded systems, while the 30% systems, which had a macroscopic crystalline appearance, yielded a Tg of 97°C. This indicates that the drug is at least partially miscible within the matrix. However, SEM and pulsed force AFM indicated that while no evidence of phase separation was seen for the 6% and 10% systems, regions of low adhesion were apparent from the AFM that were manifest as flat crystals using SEM. The data will be discussed with reference to the predictive modelling of drug miscibility in polymers. **Conclusions**: Thermal and microscopic analysis may be used to quantify and map regions of phase separation in drug-loaded polymeric systems.

57. THE DEVELOPMENT OF A TEXTURE ANALYSIS METHOD TO ASSESS THE PHYSICAL PROPERTIES OF BOVINE VITREOUS HUMOUR

***Susan Barker*[1], Lorraine M Finlay[1], Carolyn J Teague[1] and Alan W Stitt[2], [1]School of Pharmacy, Queen's University Belfast, Northern Ireland, UK, [2]Department of Ophthalmology, Queen's University Belfast, Grosvenor Road, Belfast, Northern Ireland, UK**

Purpose. We have developed a method using the technique of texture analysis to assess the physical properties of bovine vitreous humour. Initially, we have characterised the behaviour of vitreous humour from healthy young eyes (sourced from a local abattoir) and intend to use this as a basis to assess the changes in vitreous behaviour upon incubation with sugars, as a model for the diabetic situation. **Methods.** A TA.XT2 Texture Analyser (SMS, Godalming) was used with the following settings: adhesion mode, pre-test speed 10.0 mm/s; test speed 1.0 mm/s; post-test speed 3.0 mm/s; force 5 g; time 2 s; distance 4 mm, trigger type auto-2g, 2cm diameter cylindrical acrylic probe, three replicates per sample. Values of the adhesiveness, cohesiveness and work of adhesion may be derived from the texture analysis graphs via the peak height, peak width and peak area respectively. **Results.** 49 bovine vitreous humour samples were assessed using texture analysis. The results were very reproducible, both within and between samples. Overall mean (± sd) values for the three parameters were peak height 2.21 ± 0.16 g, peak width 7.65 ± 0.57 mm, peak area 8.74 ± 1.15 g.mm. Additionally, samples that had been stored refrigerated for 1 week showed no change in these parameters, indicating that no significant degradation of the textural parameters occurred over this time. **Conclusion.** A simple, rapid method of assessing the textural parameters of bovine vitreous humour has been developed.

58. THE USE OF THERMALLY STIMULATED DEPOLARISATION CURRENT (TSDC) SPECTROSCOPY TO STUDY THE GLASS TRANSITIONAL BEHAVIOUR OF POLYETHYLENE GLYCOL (PEG) 6000

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Purpose. The assessment of the glass transition (*Tg*) of semi-crystalline materials such as PEGs is difficult, due to the small transitions observed and the variable contributions of crystalline and amorphous components. TSDC is a novel and powerful technique for studying molecular mobility, which may be of use in this type of work. Here we have assessed the ability of TSDC to characterise the glass transitional behaviour of PEG 6000 and, additionally, to elucidate the effect of cooling rate on the measured *Tg*. **Methods.** PEG 6000 was supplied by Sigma Chemical Company, MO. Global TSDC experiments were performed with a TSC/RMA 9000 (TherMold Partners LP, CT) as follows: screw electrode, polarisation field 100 V/mm, polarisation temperature 70°C, polarisation time 2 minutes, lowest temperature -80°C, holding time at -80°C 2 minutes, heating rate: 10°C/minute, final temperature 20°C. The cooling rates used were 2, 5, 10 and 15 °C/minute. **Results**. The TSDC spectrum of PEG 6000 showed a single clear peak, with an extremely good signal-to-noise ratio. The temperature at the maximum peak height was between -20°C and -30°C, commensurate with previously quoted values of the *Tg* of PEGs. The peak temperature varied with cooling rate, a high cooling rate giving rise to a lower peak temperature. Very fast cooling rates gave rise to an additional negative secondary peak. It is likely that the main peak is a reflection of the glass transition of the amorphous PEG 6000. **Conclusion**. This study has shown that TSDC is capable of detecting the *Tg* of a model semi-crystalline material, PEG 6000, with great sensitivity.

59. EFFICIENT AND CONTROLLED PREPARATION OF MODIFIED DEXTRANS AS VEHICLES IN ORAL DRUG DELIVERY OF POORLY-WATER SOLUBLE DRUGS

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Purpose. To prepare hydrophobically-modified (HM) dextrans of high purity under controlled conditions, in order to create nanosized host for poorly-water soluble drugs administered orally. **Methods.** Dextran T10 (DEX; M_w = 10 kDa) was hydrophobically-modified by grafting poly (ethylene glycol) cetyl ether (PEG-C_{16}; $C_{16}EO_{10}$) nonionic surfactant. PEG-C_{16} was activated by tosylation at the terminal hydroxyl groups and coupled with dextran hydroxyl groups *via* a Williamson ether synthesis. The resulting DEX-*g*-PEG-C_{16} copolymers were purified by a dichloromethane soxhlet extraction to remove all the free PEG-C_{16} residues. The level of PEG-C_{16} grafting was determined using ^{1}H-NMR spectroscopy. The physico chemical properties of the polymeric micelles formed in aqueous solutions of DEX-*g*-PEG-C_{16} copolymers were examined using fluorescence spectroscopy and dynamic light scattering (DLS). **Results.** The tosylation modification performed using the joint action of the two amines (Et_3N and Me_3N.HCl) allowed us to achieve the tosylation of PEG-C_{16} with good yields. The ^{1}H-NMR spectroscopy shows that copolymers with different molar content in PEG-C_{16} (6-15 mol %) were obtained, depending on the ratio of the starting materials. The degree of substitution of DEX-*g*-PEG-C_{16} was calculated as $I_{Me}.100/I_a$, where I_{Me} is the average integral of the signal due to the terminal methyl protons of the PEG-C_{16} groups (~0.85 ppm) and I_a is the integral of the signal due to the anomeric protons of dextran (~4.9 ppm). In aqueous solution, HM-dextrans form polymeric micelles. The critical association concentration (CAC) was determined to be ca 4 mg/L. The size distribution of DEX-*g*-PEG-C_{16} polymeric micelles was monomodal with small average diameter (11 ± 5 nm) as determined by DLS measurements. **Conclusion.** Highly purified DEX-*g*-PEG-C_{16} copolymers, with different levels of grafting, were synthesized from biocompatible components. In aqueous solution, these copolymers form micelles with low onset of micellization and relatively small micelle mean diameters.

60. APPLICATIONS OF TISSUE ENGINEERING FOR DERMOPHARMACEUTICAL FORMULATION TESTING

***Roxane Pouliot,* Faculté de pharmacie, Université Laval, Québec, Québec, Canada**

Purpose: The aim of skin reconstruction is to come as close as possible to the normal human skin. However such an enterprise do reveal itself of an extremely high complexity. **Method:** We have developed an *in vitro* model of human skin equivalent and characterized its histologic phenotypic properties *in vitro* and *in vivo*. The dermal equivalent was made from superimposed fibroblast sheets obtained after culturing dermal cells with ascorbic acid. Then keratinocytes from newborn or youth skin were added on top to produce skin equivalents. Ten days after keratinocyte seeding, skin equivalents were either cultured at the air liquid interface or grafted on athymic nude mice. **Results:** After maturation *in vitro*, skin equivalents exhibited a well developed epidermis (basal, spinous, granular and corneum layers) expressing differentiated markers (filaggrin, transglutaminase, keratine 10) and basal membrane proteins (laminin, type IV and VII collagen). These skin equivalents were also analyzed after maturation *in vivo*. Four days after graft-

ing, the percentage of graft take over the total surface area grafted was similar in animals that received skin equivalents produced with both types of keratinocytes. Histological analysis revealed that the newly generated epidermis of newborn skin equivalents was thicker than that of youth skin equivalents. However, 21 days postgrafting, the thickness of the epidermis and the percentage of graft take were similar for both types of skin equivalents. Keratinocyte labeling with anti-HLA,-A,-B,-C confirmed that the epidermis was composed of human keratinocytes. The basement membrane components, bullous pemphigoid antigens, laminin and type IV collagen were detected at the dermo-epidermal junction, showing a continuous line 4 and 21 days postgrafting. Ultrastructural studies revealed that the basement membrane was continuous and well organized 21 days after transplantation. **Conclusion:** Therefore, these skin equivalents matured *in vitro* or *in vivo* provide new tools for pharmacological, cosmetological and toxicological testing.

61. APPLICATION OF EXPERIMENTAL CENTROID MIXTURE DESIGN FOR THE DEVELOPMENT OF EXTENDED RELEASE DEXTROMETHORPHAN HBR TABLETS.

Hatim Samir AlKhatib, Satinder Singh Bharaj and *Adel Sakr,* Industrial Pharmacy Graduate Program, University of Cincinnati Medical Center, Cincinnati, Ohio, USA

Objective: To apply experimental centroid mixture design to evaluate and optimize the effect of Polyvinyl Pyrrolidone – Polyvinyl Acetate (PVP-PVA) copolymer and two other excipients on the release of Dextromethorphan HBr (DMHBr) from controlled release matrix tablets. **Methods:** A 9-point centroid mixture experimental design was used to evaluate the effect of PVP-PVA (Kollidon^{Ò} SR) in combination with Microcrystalline Cellulose (MCC) and Dibasic Calcium Phosphate (DCP) on DMHBr release. Fractions of the excipients PVP-PVA, CMC and DCP used in the formulation were investigated. The matrix tablets were manufactured using an instrumented Manesty rotary tablet press at a constant compression force of 1000 Lb. Tablets physical characteristics and in vitro drug release were tested. Percentage release values at 1, 4 and 8 hours, apparent drug release rate and tablet hardness were used as response variables for statistical analysis. Statistical models were fitted to the data obtained by the lattice Scheffe method and multiple linear regressions. Three–dimensional response surfaces were developed to provide a visual confirmation of the nature of the experimental results and their trends. Drug release from the optimized batch was compared to the marketed German capsule product using the F_2 similarity test. **Results:** Quantitative statistical models for DMHBr release and tablet properties as a function of the mixture components were developed. Regression analysis indicated a good fit of the models. The release profile of the optimized formula was found to be similar to the marketed product according to the F_2 test. **Conclusion:** Centroid mixture design was applied successfully to the development and optimization of controlled release matrix tablets of DMHBr. PVP-PVA efficacy as a rate-controlling polymer in controlled release matrix tablets was found to be affected significantly by the type and amount of other fillers in the formulation.

62. DEVELOPMENT OF ONCE-A-DAY PROPRANOLOL HCL TABLETS

***Adel Sakr* and Elena Draganoiu, Industrial Pharmacy Graduate Program, University of Cincinnati, Cincinnati, Ohio, USA**

Purpose. To develop once-a-day Propranolol HCl matrix tablets using KollidonÒSR. **Methods.** Effect of KollidonÒSR in combination with EmcompressÒ/EmcocelÒ90M or EudragitÒ L100-55 on tablet properties and drug release was studied for the development of Propranolol HCl extended-release tablets. Directly compressible formulations were manufactured using an instrumented Manesty rotary press and the resulting tablets were tested for physical characteristics and USP drug release in various dissolution media (deionized water, pH 6.8 buffer, 0.1N HCl). The results were compared to the leading market product InderalÒLA (capsule). **Results.** It was found that KollidonÒSR at concentrations of 40-70% of the tablet weight extended the release of Propranolol in all the dissolution media, independent of compression forces above 2000lbs. KollidonÒSR contains no ionic groups and is therefore inert to drug substances. Comparing the dissolution profiles of Propranolol tablets at each polymer level in the acidic and buffer media it was observed that the release rates were similar (F2>50), but slower than the release in water, which reflected the higher solubility of Propranolol HCl in water compared to the other media. The release of Propranolol was extended up to 24 hours at KollidonÒSR concentrations of 65-70% of the tablet weight in combination with only 5% EudragitÒL100-55 or EmcompressÒ/EmcocelÒ90M. The in vitro release in the acidic stage of the optimized formulation was close to the USP limits for extended-release Propranolol capsules. The F2 test for the optimized Propranolol HCl matrix tablet and InderalÒLA showed similarity of the dissolution profiles in the buffer stage (F2>50). **Conclusions.** Using KollidonÒSR it was possible to develop once-a-day extended-release Propranolol HCl tablets which met the USP requirements. A pharmacokinetic study in healthy volunteers is currently in progress to evaluate the bioavailability of the optimized formulation and its bioequivalence to InderalÒLA; and the results will be reported.
Keywords. Tablets, Propranolol, KollidonÒSR.

63. STUDY OF THE EFFECT OF POLYVINYL ACETATE-POLYVINYLPYRROLIDINE AND FILLER

EXCIPIENTS ON THE RELEASE OF BUMETANIDE FROM EXTENDED-RELEASE, MATRIX TABLETS

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Purpose: To evaluate the effects of a novel extended-release polymer polyvinyl acetate-polyvinylpyrrolidine (PVP-PVA) and filler excipient type, dibasic calcium phosphate (DCP), microcrystalline cellulose (MCC), or spray-dried lactose (Lactose), on the release of bumetanide from extended-release, matrix tablets prepared by direct compression. **Methods**: A D-optimal mixture experiment consisting of 20 runs with 5 replicates was applied for purposes of the study. Formulations were prepared consistent with the design specifications. The formulation components were screened through 35 mesh, PVP-PVA and bumetanide were geometrically blended in a mortar and pestle, and then bulk blended with the remaining excipients in a Turbula mixer for 25 minutes. Subsequently, the respective blends were compressed on a Manesty D3B rotary tablet press to a hardness of 10 Kp. After compression, the tablets were evaluated for dissolution in a Vankel dissolution tester (37°C, 1000 mL USP purified water, USP apparatus 2, 50 rpm). Dissolved bumetanide was detected by an F-2500 Hitachi Fluorescence Spectrophotometer. The amount of bumetanide released at 1, 4, 8, and 12-hours were considered as the response variables. **Results**: The results indicate that both the extended-release polymer and excipients significantly affected bumetanide release. As PVP-PVA concentrations increased, dissolution rates declined. However, at low concentrations of PVP-PVA and high concentrations of MCC the tablets immediately disintegrated and provided no sustained release. In contrast, at low concentrations of PVP-PVA and high concentrations of DCP, release of bumetanide was extended for greater than 24 hours. Formulations containing 20% PVP-PVA, 39% DIP, and 39% Lactose provided consistent, reproducible 12-hour release of bumetanide. **Conclusions**: Both the concentration of PVP-PVA and filler excipients affected the rate of dissolution of bumetanide from extended-release, matrix tablets. By adjusting these polymers, various release profiles can be obtained. MCC concentration must be monitored closely and PVP-PVA concentration adjusted accordingly, in order to avoid rapid tablet disintegration.

64. PHARMACODYNAMIC MODELING OF SPIROMETRY MEASUREMENTS AFTER THE INHALATION OF SALBUTAMOL IN ASTHMATIC PATIENTS

Jean-Francois Marier**, Jean Lavigne, Diane Potvin, and Murray P. Ducharme**

Purpose: The bioequivalence (BE) between two formulations of salbutamol in asthmatic patients is difficult to demonstrate since plasma concentrations are virtually undetectable. The main objective of this study was to develop a novel population approach for the pharmacodynamic (PD) modeling of spirometry measurements to assess the therapeutic equivalence between two formulations of salbutamol. **Methods**: A total of 60 mild to moderate asthmatic patients received a placebo, 1, and 2 actuations of two formulations of salbutamol. Forced expiratory volume in one second (FEV_1) and forced midexpiratory flow rate ($FEF_{2575\%}$) were measured over 6 hours. An E_{MAX} function linked to a one-compartment model with relative rate (KA_{REL}) and extent of bioavailability (F_{REL}) in lungs was developed for the PD modeling of spirometry using NONMEM. Parametric point estimates with 90% confidence intervals (CI) were used to assess BE between the two formulations. Non-parametric CI was also constructed using a bootstrap resampling approach. **Results**: Population modeling of FEV_1 resulted in KA_{REL} (Mean: 1.08, CI: 1.071.16) and F_{REL} (Mean: 0.98, CI: 0.91-1.05) within BE limits with an E_{MAX} of 0.84 L/s and ED_{50} of 21.6 mg. For $FEF_{2575\%}$ data, KA_{REL} (Mean: 1.01, CI: 0.861.19) and F_{REL} (Mean: 0.92, CI: 0.86-0.99) were also within BE limits with an E_{MAX} of 1.22 L/s and ED_{50} of 39.2 mg. Covariate analyses revealed a positive correlation between height and E_{MAX} for FEV_1 and a negative correlation between age and E_{MAX} for $FEF_{25\text{-}75\%}$ data. Nonparametric point estimates and CI were similar or larger than those obtained with parametric methods. **Conclusion**: A population approach with a novel E_{MAX} model was developed for the PD modeling of salbutamol. This model was used to estimate BE with robustness in terms of rate and extent of bioavailability in lungs with the use of PD data only.

65. ESTIMATION OF ORAL ABSORPTION OF GLIBENCLAMIDE: THE USE OF *IN VITRO* AND *IN SILICO* TOOLS TO DEVELOP STRONG *IN VITRO/ IN VIVO* CORRELATIONS

Hai Wei, **Hadi Valizadeh, Raimar Loebenberg, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada**

Purpose: The purpose of this study is to predict oral performance of a class II drug, glibenclamide using *in vitro* biorelevant dissolution methods, drug permeability studies and computational technology based on biopharmaceutical drug classification system. **Methods:** Two brands of glibenclamide tablets were used. The *drug release* was tested using an Erweka DT 6 dissolution tester using different dissolution media. *Cell culture*: Caco-2 cells are cultured in 6 trans well plates over 21 days. The electrical resistance of the cell monolayer is determined using an EVOM epithelial voltmeter. The permeability test is performed if a TEER value of about 400-600 W/cm^2 is reached. *Computer simulations*: GastroPlus™ uses the advanced compartmental absorption and transit model (ACAT) for estimating the fraction dose absorbed based on permeabil-

ity and solubility parameters. *IVIVC:* The results of the simulations were compared with actual clinical data taken from literature. **Results:** The tablets of glibenclamide exhibit significant difference in their dissolution behavior depending on the nature of the dissolution media. Human effective permeability using Caco-2 cells determined to be around 3.5e-4 cm/sec. The ACAT model successfully predicted the AUC and C_{max} for most of the volunteers with less than 10% error for the two used formulations. This falls within the FDA criteria of ±15% absolute prediction error of each formulation and ±10% absolute prediction error for AUC and C_{max}. **Conclusion:** Our proposed IVIVC model exhibited excellent predictive performance for each individual plasma time curve. *In vitro/in silico* methods are a powerful tool to establish *in vitro/in vivo* correlations. The dissolution results can be used to establish strong IVIVCs which may be used as a surrogate for clinical studies.

66. DEVELOPING A NANOPARTICLE-BASED VECTOR FOR DRUG TARGETING

Yuan Huang, **Shirzad Azarmi, Raimar Loebenberg, Faulty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada**

Purpose: The goal of the project is to develop a safe and efficient nanoparticles-based carrier. **Methods**: A two-step desolvation process was used to synthesize nanoparticles from gelatin. In brief: 1.25 g of gelatin was dissolved in 25 mL of water. 25 mL of acetone was added. The high molecular weight fraction of gelatin separated as sediment to the bottom of the beaker. Then the sediment was dissolved in 25 mL of water. The pH of the solution was adjusted to pH 2.5 followed by the addition of 50- 75 mL of acetone drop-wise until nanoparticles were formed. Glutaraldehyde was added as cross linker to stabilize the nanoparticles. The important synthesize parameters such as the nature of gelatin, temperature, pH, type of desolvation agent and the effect of high shear force were investigated. The nanoparticles were labeled with Texas Red and purified using size exclusion chromatography. Confocal laser scanning microscopy (CLSM) was used for the *in vitro* determination of the cell uptake using human osteosarcoma B143 cells. **Results**: The synthesize parameters such as the nature of gelatin, pH, temperature, desolvation agent and gelatin molecular weight have important influence on the particle properties. The smallest nanoparticles 110±20 nm was produced using gelatin B at 40°C. The size of nanoparticles increased when the temperature increased. At 50°C, the size of nanoparticles was 210±30 nm, and at 60°C, the size of nanoparticles was 300±50 nm respectively. CLSM confirmed that cell uptake was achieved by nanoparticles under 200 nm. **Conclusion**: Controlling the experimental conditions nanoparticles with defined size ranges and narrow size distribution can be synthesized. The size of nanoparticles has an important influence on the cellular uptake of nanoparticles in B 143 cells.

67. SYNTHESIS AND PHYSICOCHEMICAL CHARACTERIZATION OF AMINO COPOLYMER-BASED POLYION COMPLEX MICELLES FOR THE DELIVERY OF POLYANIONIC DRUGS.

M.-H. Dufresne **(1), M. Ranger (2) and J.-C. Leroux (1), (1) Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University de Montreal, Quebec, Canada, (2) Labopharm Inc, Laval, Quebec, Canada**

Purpose. The aim of this work was to prepare and characterize copolymers presenting different pendant amino groups and to relate the pH-responsiveness of their polyelectrolyte complexes to the nature of the components. **Methods.** Polymers with different aminoethyl methacrylate monomers were synthesized by atom transfer radical polymerization (ATRP). The polymerizations were carried out at 65°C in tetrahydrofuran following activation of a poly (ethylene glycol) (PEG) macroinitiator by liganted copper (I) complexes. The polymer bearing primary amino groups required the protection of its monomers with *t*-butoxycarbonate prior to polymerization. Polymers were characterized by size exclusion chromatography and nuclear magnetic resonance spectroscopy. Micellization properties were assessed by dynamic light scattering on complexes formed from the addition of heparin to a solution of polymer. **Results.** Five PEGylated block copolymers with average-number molecular weights ranging from 4,900 to 7,400 and polydispersities lower than 1.2 were prepared by ATRP. All polymers were shown to complex heparin at optimal molar charge ratios close to 1, yielding monodispersed assemblies with sizes ranging from 25-33 nm. Complexes were shown to withstand the addition of salt at least up to physiological concentrations (i.e. 150 mM NaCl), after which they started to dissociate. All polymers were able to electrostatically interact with heparin at acidic pH. Basification of the milieu eventually led to the dissociation of the complexes, with the pH of transition being closely related to the pK_a of the copolymers. **Conclusions.** Synthesis of a PEGylated primary amine block copolymer was achieved for the first time by ATRP. Electrostatic interactions between the amino polymers and heparin triggered the formation of small, stable complexes that present great potential as drug delivery systems. NSERC is acknowledged for financial support. This work has been presented at the 2002 AAPS annual meeting and exposition on November 13. **Keywords.** Polyion complex micelles, amino copolymers, heparin

68. TEMPERATURE-TRIGGERED RELEASE OF LIPOSOMAL CONTENT USING AMPHIPHILIC POLY(ORGANO-PHOSPHAZENES)

A-C Couffin **and J-C Leroux**

Purpose. To prepare a novel family of amphiphilic temperature-sensitive poly(organo-phosphazenes) and complex them to liposomes to engineer stimuli-responsive vesicles. **Methods**. Poly(dichlorophosphazenes) were synthesized *via* controlled cationic polymerization of $Cl_3P{=}NSiMe_3$ at ambient temperature. Poly(dichlorophosphazenes) were substituted with the following side groups: ethoxyethoxyethoxy (EEE) or methoxypoly(ethylene glycol) (MPEG) and stearoyl-poly(ethylene oxide) ($C_{18}(EO)_5$) units. Absolute molecular weights were estimated by gel permeation chromatography/light scattering in either DMF/LiBr (10mM) or water/Trizma (50 mM), pH 8. The lower critical solution temperature (LCST) of the polymers (0.5 wt%) in water was determined by differential scanning calorimetry. Liposome-polymer complexes of egg phosphatidylcholine/cholesterol (EPC/Chol) (3:2 mol/mol, 30 wt% polymer) having a mean size of approximately 150 nm and loaded with the fluorescent probe, 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) and collisional quencher, *p*-xylene-bis-pyrimidium bromide (DPX), were prepared by the lipid film hydration method, followed by extrusion through polycarbonate membranes. The *in vitro* release kinetics was monitored by fluorescence spectroscopy by measuring the leakage of HPTS at different temperatures and pH 7.2. **Results**. A series of poly(organophosphazenes) with molecular weight (M_w) ranging from 20,000 to 30,000 and relatively low polydispersity (<1.3) was synthesized by living cationic polymerization. The polymers bearing EEE units and less than 6 mol% hydrophobic anchors ($C_{18}(EO)_5$) were water-soluble at room temperature. Most polymers exhibited a LCST ranging between 32 and 43°C. When complexed to EPC/Chol vesicles, the polymers were able to trigger the rapid release of liposomal contents (60%) as the temperature was raised from 30 to 50°C. Conversely, less than 5% release was observed for the control liposome formulation. **Conclusion**. Poly(organophosphazenes) with hydrophobic chains and short poly(ethylene oxide) side groups are able to permeabilize phospholipid bilayers and trigger the release of liposomal content upon an increase in temperature. These results have been presented to 2002 AAPS Annual Meeting and Exposition on November 10-14.

69. SOLUBILIZATION OF POORLY-WATER SOLUBLE DRUGS USING DEXTRAN-*G*-POLY (ETHYLENE GLYCOL) ALKYL ETHER POLYMERIC MICELLES

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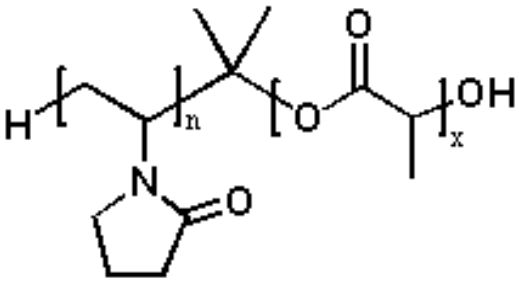
Purpose. To exploit the solubilizing potential of hydrophobically-modified dextran-*g*-poly (ethylene glycol) alkyl ether (DEX-*g*-PEG-C_n) polymeric micelles towards poorly-water soluble drugs in order to improve their oral bioavailability. **Methods.** Various hydrophobically-modified DEX-*g*-PEG-C_n graft copolymers were synthesized. Their hydrophilic backbone consisted of different molecular weight dextrans (10,000 or 40,000 Da) grafted with hydrophobic alkyl (hexadecyl or octadecyl) chains. Cyclosporin A (CsA), a highly effective immunosuppressive agent, was selected as model drug. CsA-loaded DEX-*g*-PEG-C_n micelles were prepared by a dialysis procedure using initial CsA loadings of 2.5 to 40 % (*w/w*). CsA was extracted from freeze-dried micelles using acetonitrile and assayed by high performance liquid chromatography with UV detection at 210 nm. The cytotoxicity of increasing concentrations (0 – 10 g/L) of dextran, PEG-C_{16} and DEX-*g*-PEG-C_{16} (7 mol %) towards the human colon adenocarcinoma, Caco-2, cells was evaluated using the MTT colorimetric assay. **Results.** CsA loading into DEX-*g*-PEG-C_n polymeric micelles was up to 8 times higher than ~~unmodified~~ dextrans, which have a very low affinity for CsA. The amount of incorporated CsA increased with increasing number of PEG-C_n units grafted per dextran chain and decreasing dextran molecular weight. For a constant number of PEG-C_n units (~ 2 units/dextran chain), the CsA loading achieved with DEX-*g*-PEG-C_{18} (3.9 mol%) was lower than that of DEX-*g*-PEG-C_{16} (3 mol%). On the cellular level, although free PEG-C_{16} surfactant inhibited cell growth even at concentrations below 1 g/L, DEX-*g*-PEG-C_{16} (7 mol%) polymeric micelles exhibited no significant toxicity towards Caco-2 cells, up to concentrations of 10 g/L. **Conclusion.** DEX-*g*-PEG-C_{18} micelles increase the solubility of CsA in water. The length of the hydrophilic part as well as the content and chemical nature of the hydrophobic substituents has an important effect on the solubilizing power of DEX-*g*-PEG-C_{18} .

70. PACLITAXEL-LOADED BLOCK COPOLYMER MICELLES OF POLY(*N*-VINYLPYRROLIDONE)-*BLOCK*-POLY(D,L-LACTIDE).

***D. Le Garrec*[1], *M. Ranger*[2] *and J.-C. Leroux*[1].[1]Canada Research Chair in Drug Delivery, Faculty of Pharmacy, Université de Montréal, Quebec, Canada. [2]Labopharm Inc, Laval, Quebec, Canada**

Purpose: The aim of this work was to develop a novel micellar formulation of paclitaxel (PTX) based on the amphiphilic diblock copolymer poly (*N*-vinylpyrrolidone)-*block*-poly (D, L-lactide) (PVP-*b*-PDLLA). PVP-*b*-PDLLA (Scheme 1) was synthesized using a novel procedure inspired from the work of Benahmed *et al* [1]. PTX was incorporated into polymeric micelles (PM) *via* a one-step freeze-drying process. The cytotoxicity of PTX-loaded PM was assessed *in-vitro* against different tumor cell lines and compared to that of Taxol®. Metsu : Block copolymers were synthesized by ring-opening polymerization of D,L-lactide from hydroxy-terminated PVP. PVP-*b*-PDLLA was purified by dialysis against water, centrifuged, filtered

through 0.22-mm filter and freeze-dried. The polymers were characterized by size-exclusion chromatography, MALDI-TOF, elementary analysis, thermogravimetry and ^{1}H-NMR. PTX was loaded into PM *via* a novel *tert*-butanol/water mixture lyophilization method[2], and after reconstitution, the formulation stability was monitored over time by dynamic light-scattering. Cytotoxicity of PXT-loaded PM, Taxol® and unloaded vehicles (PVP-*b*-PDLLA and Cremophor® EL) was tested *in-vitro* on OVCAR-3 human ovarian, C26 murine colon and EMT-6 murine mammary tumoral cell. **Results and discussion** : Both diblock copolymers containing 27 or 37 mol% PDLLA self-assembled in water to form 40-200 nm sized aggregates. Freeze-dried PM loaded with 2.5 or 5% (*w/w*) PTX could be readily reconstituted in sterile 5% dextrose and the formulations were stable for at least 5 h. PTX-loaded PM showed the same *in-vitro* activity than Taxol®. PVP-*b*-PDLLA was, however, less cytotoxic than Cremophor® EL. **Conclusions** : *In-vitro* data suggest that PVP-*b*-PDLLA micelles might represent a valuable alternative for formulating poorly-water soluble anticancer drugs such as PTX. References : [1] A. Benahmed *et al.* Pharm. Res. (2001) 3, 323-328. [2] D. Le Garrec *et al*. US Pat. Appl. (2002) 10/101,572.



Scheme 1. Diblock copolymer PVP-*b*-PDLLA.

71. NOVEL PH-SENSITIVE SUPRAMOLECULAR AGGREGATES AS CARRIERS FOR ORAL DELIVERY OF HYDROPHOBIC DRUGS

***V. Sant* and J-C. Leroux, Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University Of Montreal, Quebec, Canada**

Purpose: To synthesize a series of pH-sensitive amphiphilic diblock copolymers based on methoxypolyethyleneglycol-2000 (PEG) and poly (ethyl acrylate$_{50}$-*co*-methacrylic acid$_{50}$) [PEG-*b*-P (EA$_{50}$-*co*-MAA$_{50}$)] by atom transfer radical polymerization (ATRP) and evaluate solubilization of a model hydrophobic drug (indomethacin) in water. **Methods:** Four novel diblock copolymers with hydrophilic PEG block and hydrophobic poly(ethyl acrylate$_{50}$-*co*-*tert,* butyl methacrylate$_{50}$) [P(EA$_{50}$-*co*-*t*BMA$_{50}$)] block having variable molecular weight viz. 1500, 3000, 4500 and 6000 were synthesized by ATRP. These polymers were analyzed by NMR and GPC. Cleavage of *tert*-butyl groups gave corresponding PEG-*b*-P (EA$_{50}$-*co*-MAA$_{50}$). The polymers were characterized with regard to their critical aggregation concentration (CAC) and micelle size in water. Indomethacin was incorporated in the supramolecular aggregates by dialysis or O/W emulsion–solvent evaporation method. **Results:** PEG-*b*-P (EA$_{50}$-*co*-*t*BMA$_{50}$) with controlled molecular weight was obtained by ATRP. The polydispersity index as determined by GPC was < 1.27 for all polymers except 1.39 for polymer with hydrophobic block of 6,000. NMR studies indicated complete deblocking of *tert*-butyl groups giving a series of PEG-*b*-P (EA$_{50}$-*co*-MAA$_{50}$). CAC of these polymers decreased from 256 to < 80 mg/L with increase in the hydrophobic block length. Dialysis and O/W emulsion method resulted in supramolecular aggregates in size range of 270 to 340 nm and 160 to 400 nm, respectively. Polymer with hydrophobic block length of 3,000 gave maximum (5.3 %*w/w*) indomethacin loading by dialysis, whereas O/W emulsion method significantly increased drug incorporation from 5.9 to 18.3 %*w/w* with increasing hydrophobic block length. **Conclusion:** A series of PEG-b-P (EA$_{50}$-co-MAA$_{50}$) polymers with controlled molecular weight, variable hydrophobic blocks and relatively narrow polydispersity could be synthesized by ATRP. These polymers formed water-soluble supramolecular aggregates in water, and encapsulated high amounts of indomethacin. Such polymers could prove useful for the oral delivery of poorly-water-soluble drugs. This work was presented at the 2002 AAPS Annual Meeting and Exposition, in Toronto, Canada on November 10-14, 2002. Financial support from Labopharm Inc. and NSERC is acknowledged.

72. PH-SENSITIVE METHACRYLIC ACID COPOLYMERS AS ENDOSOMOLYTIC AGENTS

***MA Yessine*[1], C Meier[2], HU Petereit[2], JC leroux[1]; Canada Research Chair in Drud Delivery, Faculty of Pharmacy, Université de Montréal, Quebec, Canada[1]; Röhm GmbH, Chemische Fabrick, Werk Darmstadt, Kirschenallee, Darmstadt, Germany[2]**

Purpose. Improving the delivery of biomacromolecules into the cytoplasm requires a membrane-disruptive agent that would be capable of destabilizing membrane bilayers at the mildly acidic pH found in endosomes and would be non-disruptive at a physiological pH of 7.4. This work is aimed at characterizing five methacrylic acid (MAA) copolymers with respect to their membrane lytic properties as a function of pH. Copolymers cytotoxicity was also evaluated at pH 7.4. **Methods**. *Hemolysis.* Human red blood cells (RBC) were chosen as the endosomal membrane model. RBC (1X10^8/mL) was incubated 20 min at 37°C at pH 7.4 and 5.5 in the presence of the copolymers. After centrifugation, the release of hemoglobin was evaluated by reading the absorbance of the supernatant at 541 nm. *Cytotoxicity assay.* J774 mouse macrophage cells were incubated with the copolymers at 37°C in a humid atmosphere contain-

ing 5% CO_2. Inhibition of cell division with or without addition of a phagocytosis inhibitor (cytochalasin B) was measured using the tetrazolium salt MTT. **Results**. Two copolymers, namely poly (MAA-*co*-ethyl acrylate) (50:50 molar ratio) and poly (MAA-*co*-ethyl acrylate-*co*-methyl acrylate) (30:35:35 molar ratio), showed hemolytic activity at pH 5.5 without causing any RBC destabilization at physiological pH. Cytotoxicity assays demonstrated that the first copolymer showed significant toxicity (£30% cell survival) at low concentrations (30 mg/mL). However, the cytotoxicity of the copolymer containing 30% MAA was negligible at concentrations (³100 mg/mL) where it is highly hemolytic at pH 5.5. Although not hemolytic at neutral pH, poly(MAA-*co*-methyl acrylate-*co*-methyl methacrylate) (10:45:45 molar ratio) was very toxic on J774 cells, which was in part attributed to increased phagocytosis of this more hydrophobic copolymer. **Conclusion**. Multiple parameters influence MAA copolymer/lipid interactions at neutral as well as at acidic pH. Some of these copolymers could prove potentially useful to increase the cytoplasmic delivery of biomacromolecules. Part of this work was presented at the 2002 AAPS Annual Meeting and Exposition, Toronto, November 10-14th.

73. CHARACTERIZATION OF PH-SENSITIVE LIPOSOME/*N*-ISOPROPYLACRYLAMIDE COPOLYMER COMPLEXES

***Emmanuelle Roux*, Michel Lafleur, Émilie Lataste, Pierre Moreau and Jean-Christophe Leroux**

Purpose: To evaluate the effect of pH on the conformational changes of an N-isopropylacrylamide (NIPAM) copolymer and to assess the pH-sensitivity and pharmacokinetics of liposomes complexed with this copolymer. **Methods**: A randomly-alkylated copolymer of NIPAM, methacrylic acid and N-vinyl-2-pyrrolidone was synthesized. Phase transition of the copolymer was studied as a function of temperature and pH by differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy. pH-responsive vesicles were obtained by the complexation of the copolymer to liposomes and they were characterized with regards to pH-triggered content release, serum-stability and pharmacokinetics in rats. **Results**: DSC experiments conducted at pH 7.4 revealed that the temperature at the maximum of the endothermic peak associated with the phase transition was 35°C. The endotherm was very broad and ranged from 23 to 68°C. FTIR spectroscopy analysis showed that the polymer maintained a high level of hydration at 37°C and pH 7.4, even if the polymer already underwent its coil-to-globule phase transition. As the pH was reduced, the phase transition became more cooperative and occurred at lower temperature. Copolymer phase transition was not substantially influenced by its complexation to liposomes. The liposome/copolymer complexes were stable at 37°C and pH 7.4 but rapidly released their contents under acidic conditions. Circulation time of liposomes in rats was slightly increased by their complexation with the copolymer. A substantial enhancement of the liposome circulation time could be obtained by further addition of poly (ethylene glycol)-lipid derivative (PEG) to the formulation. However, PEG reduced the pH-sensitivity of the vesicles. **Conclusion**: This study revealed that, at neutral pH, the copolymer was not in its random coil conformation, and thus may not provide liposomes with an optimal steric protection in vivo. Although the addition of PEG efficiently prolongs circulation time, it also leads to a significant decrease in liposome pH-sensitivity.

74. NOVEL PH-SENSITIVE UNIMOLECULAR MICELLES AS ORAL DRUG DELIVERY SYSTEMS FOR LIPOPHILIC COMPOUNDS

***M.-C. Jones*[1], M. Ranger[2] and J.-C. Leroux[1];**
[1]Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University of Montreal, Québec, Canada;
[2]Labopharm Inc., Laval, Québec, Canada

Purpose: To synthesize a novel drug carrier from amphiphilic pH-sensitive star-shaped polymers. These unimolecular polymeric micelles will act as carriers for the oral administration of poorly water-soluble drugs. **Methods:** Unimolecular polymeric micelles (UPM) were synthesized by atom transfer radical polymerization (ATRP) from a four-armed initiator by a two-step polymerization procedure. First, the core was obtained from the polymerization of hydrophobic ethyl methacrylate and tert-butyl methacrylate in THF at 65°C using copper(I) bromide and N,N,N',N',N''-pentamethyldiethylenetriamine as catalyst and ligand respectively. Secondly, poly(ethylene glycol) methacrylate was added to afford an hydrophilic shell. Acidic cleavage of tert-butyl methacrylate units into methacrylic acid introduced ionisable carboxylic functions and thus, rendered the polymer sensitive to pH variations. The polymers were characterized by NMR, size exclusion chromatography and fluorescence spectroscopy. Micelle size was determined by dynamic light scattering. Drug loading assays were conducted using indomethacin as a model hydrophobic compound. Micelles were loaded using the oil-in-water emulsion method. **Results:** Star polymers of various compositions and molecular weights (Mn: 9000- 22000) were obtained. Micelle sizes were small, ranging from 10 to 30 nm, and depended on molecular weight. The effect of pH on the polarity of hydrolysed UPM was evaluated by fluorescence spectroscopy using pyrene as a probe. The results showed a decrease in the pyrene emission intensity as the pH was shifted from acidic (pH 3) to basic (pH 11), which is consistent with an increase in polarity as the carboxylic functions become ionized. Final drug loadings of 4 and 8% (w/w) were obtained with 80% entrapment efficiency. **Conclusions:** pH-sensitive unimolecular micelles can be obtained by ATRP and loaded with a hydrophobic drug at high entrapment efficiencies. *This work has previously been*

presented at the AAPS meeting in Toronto (November 2002).

75. STABILIZATION OF NANOPARTICULATE DISPERSIONS USING PROTEINS AS SURFACE STABILIZERS

***Christian Wertz*, Elan Drug Delivery, Inc., King of Prussia, Pennsylvania, USA**

Purpose. To investigate the use of surface-active proteins as surface stabilizers in nanoparticulate dispersions and to compare the bioadhesive nature of these formulations to traditional dispersions formulated with polymeric stabilizers. **Methods.** High-energy milling studies were conducted using various drug (naproxen, nimesulide, and itraconazole) and protein (fibrinogen, γ-globulin, albumin, casein, and lysozyme) combinations. Resulting dispersions were characterized using particle size analysis, microscopy, and electrophoresis. Using barium sulfate as a model drug, a stable drug/protein (lysozyme) dispersion was then compared to a traditional drug/polymer (HPC-SL) dispersion using a suitable bioadhesion assay to determine the extent of nanoparticulate adhesion to mucin substrates. **Results.** Stable nanoparticulate dispersions were generated only when lysozyme was used as the surface stabilizer. Formulations using other proteins resulted in severely aggregated, unstable systems. Electrophoresis measurements on the stable lysozyme formulations revealed large, positive zeta potentials, indicating the importance of electrostatic repulsion in these systems. The positive charge associated with lysozyme was also responsible for the strong adhesion of the nanoparticles to the negatively charged mucin layers, resulting in enhanced bioadhesion when compared to uncharged polymer systems. **Conclusions.** Due to its relatively compact size, structural stability, and high isoelectric point, lysozyme proved a suitable surface stabilizer in the formulation of stable nanoparticulate dispersions while proteins with different physical and structural characteristics performed poorly. These characteristics also resulted in improved bioadhesion of the nanoparticulate dispersions, making lysozyme an ideal surface stabilizer for the delivery of poorly soluble drugs having low bioavailabilities.

76. WOUND HEALING ACTIVITY OF HIALURONIC ACID FROM UMBILICAL CORD

***Loida Oruña Sanchez*, Gabriel Coto, Lic. Guillermo Lago Histoterapia Placentaria Center; Regulatory Authorities of Quality Control of the Drug**

The hyaluronic acid (HA) is a glucosaminoglycans present in the connective tissue of all body and have very terapeutical effect. **Purpose**. A HA crude extracted from the umbilical cord of human placentae was pharmacologically assessed as healing jelly. **Methods**. The experimental design used 20 Sprague Dawley adult rats as biological model. The environmental conditions such as air exchange cycles, room temperature, relative humidity and light cycle were controlled throughout the experiment. Four wounds were made in the dorsum to each animal using disposable cutaneous biotomes of 9 mm. Treatments applied were: control spontaneous (C), placebo (P), silver sulfadiazine (SS) and HA at 4%. Animals were treated for 7 days and there was a daily clinical control of the wounds. The samples of ten animal was processed for histophatology stained with hematoxilin/eosin and Masson´s trichrome for evaluate the dermal reconstitution and epithelial migration. The samples of the other animal were used for a morphometric study of all measurements through an imagen processing software (Digipat System, EICISOFT. Cuba), was determined the percentage of total re-epithelized area, the epithelial linear growth, the wound perimeter and circularity factor. **Results**. The product evaluated HA showed a better response regarding the remaining treatments with the healing of 70% of the wounds in the epithelial migration. Also it controlled the dermal reconstitution by 50% in the II degree and by 20% in the III degree demonstrating a matrix with collagenous fibres more mature than the rest. In the planimetry study this treatment was also of best evolution with 89.33% of re-epithelialized area, a shorter wound perimeter with 10.32 mm and larger lineal growth with 5.5 mm and a circularity lower than 61.33 for ($p< 0.05$). this results are similar a the literature publicate. **Conclusion**. The hyaluronic acid jelly at 4% is a medication that contributes to accelerate the wound healing of the biomodel essayed. Key words: re-epittelization, dermal reconstitutions, hialuronic acid, wound healing.

Delegates - (as of May 14, 2003)

Abbott	Frank	University of British Columbia	Canada
Abbott	Phyllis	-	Canada
Abreu	Luis	Universidade Estadual de Campinas	Brazil
Aghamanoukian	Tamar	Biovail Contract Research	Canada
Aghazadeh-Habashi	Ali	University of Alberta	Canada
Alibhai	Mehmood	Novartis	Canada
Allen	Christine	University of Toronto	Canada
Allport	Gérald	Ministère des Finances	Canada
Amestoy	Claude	Algorithme Pharma Inc.	Canada
Annedi	Subhash	University of Toronto	Canada
Arvanitakis	Nancy	Algorithme Pharma Inc.	Canada
Assi	Abdel-Azim	Assiut University	Egypt
Bailey	David	London Health Sciences Centre	Canada
Barker	Susan	Queen's University Belfast	United Kingdom
Bélanger	Pierre	University of Montreal	Canada
Bello-Ramirez	Angelica	University of Toronto	Canada
Bendayan	Reina	University of Toronto	Canada
Berryman	Leigh	LAB Pre-Clinical Research International, Inc.	Canada
Biro	Monica	Caprion Pharmaceuticals Inc.	Canada
Bisson	Cédric	MSBI Capital	Canada
Black	Michael	Aventis Pharma S.A.	France
Blanchette	Susan	Pfizer Canada Inc.	Canada
Blaschke	Terry	Stanford University School of Medicine	USA
Boch	Ron	QLT Inc.	Canada
Boudjikanian	Lory	Gattefosse Canada Inc.	Canada
Bös	Michael	Boehringer Ingelheim (Canada) Ltd.	Canada
Bouallegue	Ali	University of Montreal	Canada
Brocks	Dion	University of Alberta	Canada
Brunet	Jean-Sebastien	Algorithme Pharma Inc.	Canada
Bulger	Lynne	Hoffmann-La Roche	Canada
Buluran	Josie	Wyeth Research	USA
C.-Gaudreault	Rene	C.H.U.Q.	Canada
Caillé	Louis	Algorithme Pharma Inc.	Canada
Carpenter	Tom	LAB Pre-Clinical Research International, Inc.	Canada
Casley	Bill	Health Canada	Canada
Chau	Anton	University of British Columbia	Canada
Chebli	Chafic	Pharmascience Inc.	Canada
Chelsky	Dan	Caprion Pharmaceuticals Inc.	Canada
Chen	Silver	University of Toronto	Canada
Cheung	Catherine	University of British Columbia	Canada
Cheung	Francesca Ting-Yan	University of Toronto	Canada
Chow	Kwok	Patheon Inc.	Canada
Clas	Sophie-Dorothée	Merck Frosst Canada & Co.	Canada
Cloutier	Jean-François	MIT/PureCell Technologies	Canada
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Notes

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