CSPS

SYMPOSIUM 2002

New Technologies In Drug Discovery & Drug Development

CANADIAN SOCIETY FOR PHARMACEUTICAL SCIENCES

The Banff Centre, Signature Shot
Photo: Scott Rowed

The Banff Centre
Banff, Alberta, Canada
13-15 June 2002

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5th Symposium programme - New technologies in drug discovery & drug development

Wine & Cheese Welcoming Reception, Wednesday, June 12, 1900h-2030h, Foyer, Max Bell Building, The Banff Centre

Exhibitors, Thursday, Friday, June 13, 14, 0900h-1600h, Saturday, June 15, 0900h - noon, Lounge, Max Bell Building, The Banff Centre

CSPS Programme

Thursday, June 13

0800h-1600h *Poster Presentations*, MB252, Max Bell Building
The Banff Centre

0830h-1520h *Symposium,* Auditorium, Max Bell Building
The Banff Centre

0900h *Welcome*. Programme Chair: Fakhreddin Jamali, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, Canada

Session 1: *Genomic & Proteomic Concepts in Drug Discovery & Development.* Chair: Pollen K.F. Yeung, College of Pharmacy, Dalhousie University, Halifax, Nova Scotia

0905h *Proteomics: ProteinChip® Technology*. Deborah L. Diamond, Ciphergen Biosystems, Inc., Fremont, California

0940h *Pharmacogenomics and Idiosyncratic Drug Reactions*. Alastair E. Cribb, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown

1015h **Coffee/Tea Break.** Meet the poster presenters.

1045h *Unraveling Human Diversity: The Power to Convert Genetic Code into Medical Cures*. Tristan Orpin, Corporate Sales & Marketing, Sequenom Inc, San Diego, California

1120h *Pharmacogenetics: Current Possibilities and Future Trends*. Brad Popovich, Diagnostics and Pharmacogenomics, Xenon Genetics Inc., Burnaby, British Columbia

1200h **Lunch and Learn.** Private Function Rooms 3, 4, & 5, Donald Cameron Hall, The Banff Centre. *R&D: Partnership Between the CCRA and the Pharmaceutical/Biopharmaceutical Sector.* Jean Marion, Sector Specialist, Scientific Research & Experimental Development, CCRA, Ottawa, Ontario

Session 2: *Innovative Approaches to Drug Discovery & Delivery.* Chair: Antoine A. Noujaim, Altarex, Edmonton, Alberta; Éric Masson, Scientific and Regulatory Affairs, Anapharm, Québec City, Québec

1330h *Novel Drug Delivery Systems from the Rock: From Gene Therapy to Marine Pharmaceuticals*. Hu Liu, School of Pharmacy, Memorial University of Newfoundland, St. John's, Newfoundland

1410h *Immune-based Drugs: New Paradigms Challenging Conventional Drug Development Programs*. Antoine A. Noujaim, Altarex, Edmonton, Alberta

1450h *Bispecific and Bifunctional Nanoprobes: A New Generation of Diagnostics and Therapeutics*. Mavanur R. Suresh, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton

1530h **Coffee/Tea Break.** Meet the poster presenters.

1600h *CSPS Members Annual General Meeting,* Auditorium

1700h Guided Tour, privately arranged

Friday, June 14

0800h-1600h *Poster Presentations*, MB252, Max Bell Building
The Banff Centre

0830h-1730h *Symposium,* Auditorium, Max Bell Building
The Banff Centre

Session 3: *New Technologies in Drug Development.* Chairs: Elizabeth Vadas, Pharmaceutical Research & Development, Centre for Therapeutic Research, Merck Frosst Canada & Co., Montreal, Quebec, Canada; Thomas Spencer, Angiotech Pharmaceuticals, Vancouver, British Columbia

0830h *Opening Remarks*. Chairs: Elizabeth Vadas, Thomas Spencer

0845h *Water-soluble Amphiphilic Nanocarriers*. Jean-Christophe Leroux, Faculté de Pharmacie, Université de Montréal, Quebec

0920h *Drug Delivery Systems For Paclitaxel: Implantable Films And Pastes*. Helen M. Burt, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver

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

1025h *The Potential Use Of Polymers (Liposomes Or Micelles) As Drug Delivery Systems*. Adi Eisenberg, Department of Chemistry, McGill University, Montreal, Quebec

1100h *Novel Drug Delivery Approaches*. William Bosch, Dispersion Technology, Elan Drug Delivery, King of Prussia, Pennsylvania

1135h Panel Discussion

1200h **Lunch Break.**

Session 4: *New Technologies in Drug Delivery.* Chairs: Elizabeth Vadas, Pharmaceutical Research & Development, Centre for Therapeutic Research, Merck Frosst Canada & Co., Montreal, Quebec; Thomas Spencer, Angiotech Pharmaceuticals, Vancouver, British Columbia, Canada

1300h *Different Aspects Of Imaging Techniques And Their Use In Drug Development*. Timothy J. McCarthy, Experimental Medicine, Pharmacia Corporation, St. Louis, Missouri

1335h *PET/SPECT In Evaluation Of Novel Dosage Forms*. Marc S. Berridge, Department of Chemistry, Case Western Reserve University, Cleveland, Ohio, USA

1410h *Biomarker Applications of Positron Emission Tomography*. Daniel Salazar, Clinical Discovery, Bristol Myers Squibb Company, New York, New York

1445h Panel Discussion

1500h **Coffee/Tea Break.** Meet the poster presenters.

1600h ***Poster Presentations By Merck Company Foundation Summer Research Award Recipients.*** Chair, Frank Abbott, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver

1800h Dinner and awards banquet, **Banff Park Lodge**, sponsored by AstraZeneca.

Saturday, June 15

0800h-1200h *Symposium,* Auditorium, Max Bell Building
The Banff Centre

Session 5: *Biotech Drug Development Challenges.* Chairs: Iain J. McGilveray, McGilveray Pharmacon, Ottawa, Ontario; Gordon McKay, PharmaLytics Inc., Saskatoon, Sask.

0800h *Proteomics*. John J. M. Bergeron, Faculty of Medicine, McGill University, Montreal, Quebec, Canada

0835h *Vaccine Formulation, Stability and Delivery*. Carl Burke, Vaccine Pharmaceutical Research Department, Merck Research Laboratories, West Point, Pennsylvania

0910h *Inhalation Delivery Of Protein Drugs*. Lynn Van Campen, Inhale Therapeutic Systems, Inc., San Carlos, California

0945h **Coffee/Tea Break.**

1000h *Mass Spec: The Panacea - Or Not?* Alfred L. Yergey, National Institute of Child Health and Human Development, Bethesda, Maryland, USA

1035h *Comparability And Other Regulatory Issues For Biotech Drugs*. Anthony Ridgway, Health Products and Food Branch, Health Canada, Ottawa, Ontario

1110h *Challenges In Developing A Biotech Drug Product*. Leanne Embree, Analytical Chemistry, Angiotech Pharmaceuticals Inc., Vancouver, British Columbia

1145h Panel Discussion

1200h **Closing:** Concluding Remarks
Elizabeth Vadas, President, CSPS

Programme Committee

Fakhreddin Jamali, University of Alberta, Alberta (Chair)
Éric Masson, Anapharm, Inc., Quebec
Iain McGilveray, McGilveray Pharmacon Inc., Ontario
Gordon McKay, Pharmalytics Inc., Saskatchewan
Ronald Reid, University of B.C., British Columbia
Thomas Spencer, Angiotech Pharmaceuticals, B.C.
Elizabeth Vadas, Merck Frosst Canada Inc., Quebec
Kishor Wasan, University of B.C., British Columbia
Pollen Yeung, Dalhousie University, Nova Scotia

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Welcome Address

Elizabeth Vadas
President of CSPS

Elizabeth B. Vadas obtained her PhD. in Physical Chemistry from McGill University. Following postdoctoral training she joined Merck Frosst in 1980 as a senior research scientist in the department of Pharmaceutical Research and Development. Over the years she has been involved in the development of many new chemical entities discovered at the Merck Frosst Centre for Therapeutic Research while taking on increasing management responsibilities. Currently Dr. Vadas is Executive Director of Pharmaceutical Research and Development, a department which has grown from 18 to over 80 scientists in the last 10 years under Dr. Vadas' leadership. Most notable of her department's scientific accomplishments are the product development efforts supporting the leukotriene and Cox-2 programs leading to the regulatory approval of SINGULAIR® in the former and VIOXX® in the latter; and to the regulatory submission of ARCOXIA™, Merck's second coxib. Dr. Vadas' main scientific interests are in the area of pharmaceutics, particularly in solid state chemistry and physics, drug excipient interactions and aerosols. She is an Adjunct Professor of Pharmaceutics at the Faculty of Pharmacy, University of Montreal. She also has lectured and published widely. Professional activities and honours:

- USP Aerosol Advisory Panel member 1989-1990, 1992-1995
- USP Committee of Revision member 1990-1995
- Louis W. Busse lecturer, School of Pharmacy, University of Wisconsin, 1991
- Elected Fellow of American Association of Pharmaceutical Scientists 1996
- "Nouveaux Performants" Management Award 1998
- Elected to the Executive of CSPS as Member-at-Large (1998-2000)
- Canadian Health Manager Award 1999
- CSPS DuPont Leadership Award 2000
- Elected President CSPS in 2001

Overview of the conference

Fakhreddin Jamali
Chair of the Programme Committee

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 20 PhDs. He is 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. 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.

SESSION 1

Genomic & proteomic concepts in drug discovery & drug development

Introductory remarks

Pollen Yeung
Professor and Director, Pharmacokinetics and
Metabolism Laboratory, College of Pharmacy,
Department of Medicine, Faculties of Health
Professions & Medicine, 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 (BSc. Pharm. 1979) and MSc. (1982) from the University of Manitoba, Winnipeg, Manitoba, and PhD. in pharmaceutical chemistry from the University of Saskatchewan (1986), Saskatoon, Saskatchewan. He joined the College of Pharmacy, Dalhousie University, 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 and metabolism of cardiovascular drugs, and their effects on hemodynamic and neurohormone regulation that 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, which is an industry association promoting biotechnology and life sciences industry in Nova Scotia and Atlantic Canada, and also a member of the executive council for the Canadian Society of Pharmaceutical Scientists.

Proteomics: ProteinChip® Technology

Deborah Diamond Ciphergen Biosystems, Fremont, CA

Direct DNA sequencing and mRNA expression profiling methodologies have provided a wealth of genomic information that has been used to address needs in the diagnosis and treatment of disease. In this regard, differential gene display is at the core of many efforts aimed at correlating transcription profiles with phenotypic differences (i.e. metabolic disease, cancer, microbial virulence) and plays a key role in many drug discovery programs. Several observations suggest however, that studies aimed at discovery of new drug targets based solely on genomic efforts are not optimal. Increased transcription of a gene does not necessarily correlate with higher protein levels, differentially modified targets will be missed by this approach and, there is no identifiable mRNA source to mirror differential expression of surrogate protein biomarkers secreted into biological fluids (i.e. serum, urine, cerebrospinal fluid). In combination with genomics, proteomics efforts involving differential analysis, identification, and characterization of proteins will provide a more comprehensive approach to the development of new drug intervention strategies. Ciphergen ProteinChip Array, Surface Enhanced Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (SELDI-TOF MS) technology represents a novel retentate chromatography system that fully integrates processing of proteins, including separation, detection and various protein function analyses on a single biochip platform. Crude biological samples (cell or tissue extracts, physiologic fluids, etc.) are incubated directly on SELDI ProteinChip Arrays containing chemically (ionic, hydrophobic, hydrophilic, etc.) or biochemically (antibody, receptor, DNA, etc.) treated surfaces for specific protein interactions. After extraction upon the SELDI surface, the retained proteins are detected by laser desorption ionization time-of-flight mass spectrometry. These conventional chromatography and biologically based SELDI surfaces can be used in a combinatorial fashion to profile changes in protein expression patterns for studies aimed at tracking disease onset, disease progression, therapeutic efficacy, and toxicity monitoring. A key feature of the technology is the ability to rapidly screen many samples in parallel to validate the presence of multiple biomarker candidates. This presentation will provide an overview of the technology and examples from several applications relevant to protein-targeted drug discovery.

Deborah L. Diamond
Ciphergen Biosystems, Fremont, California, USA

Dr. Diamond is a senior field research scientist at Ciphergen Biosystems, Inc. She received her PhD in Biochemistry from the University of Massachusetts Medical School, Worcester, MA USA (1993) where her research interests centred on studying the metabolic regulation of glucose transport. She was the 1992 recipient of the Marios C. Balodimos Award for Scientific Research presented by the American Diabetes Association at the 31st Annual Scientific Meeting of the A.D.A., Massachusetts Affiliate. She then received her postdoctoral training in Biochemistry and Biophysics at Washington State University, Pullman, WA USA (1993-1998) where her research interests included protein folding and export, characterization of the mechanism of action of molecular chaperones, and protein characterization and elucidation of structure-function relationship. In 1998, she joined Ciphergen Biosystems as a field research scientist for the Pacific Northwest and was promoted to senior scientist in 2000. Her current scientific efforts are aimed at using ProteinChip® arrays and time-of-flight mass spectrometry for differential protein expression profiling; protein purification, characterization, and identification; protein-protein interactions; and protein-nucleic acid interactions.

Unraveling Human Diversity: The Power to Convert Genetic Code into Medical Cures

Tristan Orpin, VP, Corporate Sales and Marketing,
SEQUENOM 3595 John Hopkins Court, San Diego CA

The most effective strategy to find the genes associated with major human diseases will combine elements of a candidate gene approach with the use of economical whole genome scans. This mixed approach will in turn require the integration of four essential components:

- An accurate and ultra-high throughput genotyping technology
- Access to an extensive panel of validated genotyping assays or the ability to automate the efficient design and implementation of such a panel
- The rapid determination of allele frequencies in populations of several hundred individuals
- Well-qualified and stratified DNA banks

Details of how this strategy is being successfully applied using an automated genotyping platform based on a primer extension reaction monitored by MALDI-TOF mass spectrometry will be reviewed. Results will be presented verifying that in an age-stratified population of healthy individuals, disease associated SNPs can be identified by their frequency reduction as age increases.

Tristan Orpin
VP, Corporate Sales and Marketing, SEQUENOM,
San Diego , California, USA

Tristan Orpin is currently the Vice President of Sales and Marketing at SEQUENOM Inc. a San Diego based company engaged in genomics research and the development of industrial-scale solutions for high throughput genotyping. Tristan has global responsibilities for market development and support of the company's genotyping technologies. Prior to joining SEQUENOM, Tristan was an International Marketing Manager at Bio-Rad Laboratories (Hercules, CA). He has held numerous other commercial roles including that of Life Sciences General Manager for Bio-Rad Oceania. Tristan graduated with first class honours from the University of Melbourne, Australia and has held research positions at the University of Melbourne and the Walter & Eliza Hall Research Institute.

Pharmacogenetics: Current Possibilities and Future Trends

Brad Popovich, Senior Director, Diagnostics and Pharmacogenomics, Xenon Genetics Inc., 3500 Gilmore Way, Burnaby, BC V5G 4W8

Adverse drug reactions (ADRs) are a major medical and economic problem in the health care industry. ADRs are reported to be the fifth leading cause of death in the US, and account for approximately 7% of all hospitalizations. The social and economic burden associated with ADRs is tremendous, and society is in need of medical tests to allow individuals at-risk for ADRs to be screened prior to receiving drug therapies. Pharmacogenetics (PGx) is heralding a new era in therapeutic intervention and drug development by allowing the detection of DNA-based genetic variation, which plays a role in determining an individual's unique response to drug therapy. PGx will lead to the development of genetic tests capable of detecting such variation, and these tests will clearly play a prominent role in determining specific drug efficacy and patient predisposition to ADRs. Presently two general approaches are being used in genetic studies aimed at developing PGx tests: these include candidate gene and whole genome association studies. The candidate gene approach is based on selecting genes known or suspected to be involved in biological activities related to the drug under evaluation. This approach is primarily useful in selecting genes related to drug metabolism (e.g. cytochrome p450s), but it has proven to be ineffective in the selection of genetic targets not involving drug metabolism. The whole genome approach in contrast is not based on prior knowledge of candidate genes, but this "genomic" approach has not been successful in PGx due to numerous factors and in particular the lack of statistical power in genetic association studies using unrelated individuals, and general signal to noise problems associated with genes conferring low relative risks for the phenotype of interest (i.e. the ADR). What is clearly needed is a genetic approach to identify novel genes underlying ADRs, however the necessary family studies are impossible to perform since the ADR phenotype is only seen when the respective drug is administered to a patient. This fact precludes performing family studies since related family members are at-risk for the ADR if a genetic predisposition is assumed making such studies unethical. The resulting lack of a genetic model system to study the genetics of ADRs is the single most important factor that has limited the application of this technology in the practice of medicine to date. Xenon Genetics is presently developing a model system using recombinant congenic for the evaluation of ADRs and drug efficacy. To identify novel genes causing predisposition to ADRs, Xenon is designing a comprehensive genetic discovery platform based on the use of both inbred and recombinant congenic strains of mice. This talk will summarize the application of this platform to the discovery of genes related to drug efficacy and ADRs.

Brad Popovich

Senior Director, Diagnostics and Pharmacogenomics,
Xenon Genetics Inc., Burnaby, British columbia,
Canada

Brad Popovich is presently the Senior Director of Pharmacogenomics and Diagnostics at Xenon Genetics Inc. (Vancouver, British Columbia). He has been with Xenon Genetics since June 2001. Previously, Popovich was at Oregon Health Sciences University (OHSU) where he was a tenured professor in the Department of Molecular and Medical Genetics. His research was focused on the characterization of the mechanism of expansion of the CGG repeat in the fragile X syndrome, and the development of molecular assays for a wide variety of genetic diseases. His clinical responsibilities were as the Director of the OHSU Clinical Genetic Laboratories, and the DNA Diagnostic Laboratory in the University Hospital. In addition, Popovich was also the Director of Graduate Education in the Department of Molecular and Medical Genetics at OHSU. Popovich is a presently a board member and Vice President of the American College of Medical Genetics (ACMG) where he chairs the Lab Practice Committee. He is the Co-chair of the ACMG / College of American Pathologists Biochemical and Molecular Genetics Resource Committee, which is the committee responsible for the external quality assessment (EQA) programs in both biochemical, and molecular genetics. This is the largest EQA program worldwide for clinical laboratories offering genetic testing serving over 200 labs in 11 countries. In addition to Popovich's academic interests in genetic testing, he has been involved in the evolution of DNA based forensic testing. He was the prosecutions DNA expert in the OJ Simpson matter and the plaintiffs DNA expert in the Simpson civil case. He has also been involved in numerous other forensic cases and has testified for both the prosecution and defense throughout the US and abroad. Popovich co-authored the first comprehensive genetic privacy law in the US, and helped get this legislation signed into law in Oregon in 1995. He then chaired the Oregon Genetic Privacy Advisory Committee, and testified regarding proposed amendments to this law in the Oregon Senate and House during the 1997, 1999, and 2001 legislative sessions. Popovich received his PhD in molecular genetics and MSc in biochemical genetics from McGill University, MS in genetic counseling from Sarah Lawrence College, and BA in biology from Oakland University. He completed his postdoctoral and clinical genetic fellowship training at the University of Wisconsin, and University of North Carolina. His first post-training position was as the founding Director of Molecular Genetics at Children's Hospital San Diego

R&D: Partnership between the CCRA and the Pharmaceutical / Biopharmaceutical Sector

Jean Marion, Canada Customs and Revenue Agency

The Canada Customs and Revenue Agency (CCRA) released two policy documents concerning the pharmaceutical and biopharmaceutical sector in May 2001. The first document is entitled "Application Policy 96-09R - Eligibility of Clinical Research in the Pharmaceutical Industry" and the second, "Application Policy 2001-02 - Multinational Clinical Trials". These policies address the eligibility of clinical research work claimed under the Scientific Research and Experimental Development (SR&ED) tax incentive program. As an initial step in preparing these documents, the CCRA invited the industry to establish a sector taskforce. The presentation outlines the co-operative effort of the CCRA and the Industry Taskforce in finding solutions to eligibility issues. It also examines the principles, on which the Application Policies are based, the approach to the eligibility of work performed by Canadian claimants in Canada as part of multinational clinical trials and the revisions made with respect to Phase IV clinical trials, pharmacoeconomics and epidemiology. In addition, sources of supporting information that can be used to substantiate the work being claimed under the SR&ED program are discussed.

Jean Marion
Sector Specialist, Scientific Research &
Experimental Development, Canada Customs and
Revenue Agency, Ottawa, Ontario, Canada

Jean Marion joined the Canada Customs and Revenue Agency in July 1999 as a National Technology Sector Specialist within the Scientific Research and Experimental Development Program. He is working with the Pharmaceutical and Biotechnology Sectors. Jean is seconded from Canada's Research-Based Pharmaceutical Companies (Rx&D). Previously, he managed clinical studies at Merck Frosst Canada as a Clinical Research Associate. Prior to joining the private sector, Jean was part of a team at Industry Canada working to improve the business environment affecting the pharmaceutical industry. His career in government also included a posting to the Canadian Embassy in Brussels as a Science and Technology Counsellor and a role in the implementation of the University-Industry Program at the Natural Sciences and Engineering Research Council (NSERC). Jean received a doctorate degree in biochemistry from McGill University in 1980 and worked as an Industrial Research Fellow at Ayerst Laboratories and Bioresearch Laboratories.

SESSION 2

Innovative approaches to drug discovery & delivery

Eric Masson
Senior Director, Scientific and Regulatory Affairs,
Anapharm Inc., 2050 boul. Rene-Levesque W,
Quebec, QC, Canada G1V 2K8

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. Now Senior Director of Scientific and Regulatory Affairs at Anapharm-member of SFBC International, he is leading a team responsible for scientific writing, Biometrics, and Regulatory Affairs. Dr Masson still maintains relationship with academia, lecturing courses to graduate students in drug development, and as external reviewers for Ph.D. thesis. Éric is also Vice-President of the Canadian Society of Pharmaceutical Scientists. He has authored several scientific papers.

Novel Pharmaceuticals from the Rock: From Gene Therapy to Marine Pharmaceuticals

Hu Liu, School of Pharmacy, Memorial University of Newfoundland, St. John's, NF, Canada

Gene therapy addresses the root causes of many genetically inherited and environmentally caused diseases. Cationic liposomes are non-viral vectors studied. However, liposomal-mediated transfection levels in the liver are significantly lower than those observed in other organs such as the lungs. We have been studying the delivery of p53 gene and low-density lipoprotein receptor (ldlr) gene to the liver. It is known that about 40% of liver cancer patients have either missing or mutated p53 genes. It is believed that introducing functional p53 gene will cause apoptosis (programmed cell death) in hepatoma cells. In familiar hypercholesterolemia (FH) patients, ldlr gene in the liver is either missing or nonfunctional which results in elevated cholesterol level in the blood. It is apparent that safe and efficient delivery of functional ldlr gene to the liver will reduce the cholesterol level, especially LDL associated cholesterol. In our effort of delivering these two important genes, p53 and ldlr gene, to the liver cells, a novel peptide with strong affinity for liver cells has been designed and synthesized. This peptide was evaluated for its liver targeting potential for liposomal-mediated gene delivery. Newfoundland being located in North Atlantic Ocean is a great source of many unique cold marine biomaterials. We have been actively working on the development of marine based pharmaceuticals including w-3 oil based lipid emulsions.

Hu Liu
School of Pharmacy, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

Dr. Hu Liu is an Associate Professor of Pharmacy and holds cross-appointments to the Department of Biochemistry and the Division of Basic Medical Sciences at Memorial University of Newfoundland, St. John's, NF, Canada. He is a recipient of Rx&D-HRF/CIHR Career Award in Health Sciences (1999-2004) and was the winner of the 2000 AFPC Astra-Zenecca Young Investigator Research Award. Dr. Liu received his B.Sc. (Pharm) and M.Sc. (Pharmaceutical Chemistry) degrees from Beijing Medical University, Beijing, China. He came to Canada in 1987 and studied first in Chemistry and later in Biochemistry at the University of Alberta, Edmonton, AB, Canada. After finished a Ph.D. degree in lipoprotein biochemistry in 1993, he did a post-doctoral training under the mentorship of Professor Raymond Counsell of the Departments of Pharmacology, Medicinal Chemistry and Radiology at the University of Michigan, Ann Arbor, MI, USA before returning to Canada to take a faculty position at Memorial University of Newfoundland in 1994. His research interests include drug/gene targeting, development of diagnostic agents for early detection of cancer and atheroma, and development of cold ocean derived pharmaceuticals.

Pharmacogenomics and Idiosyncratic Drug Reactions

Alastair Cribb, PEI Health Research Institute and Atlantic Veterinary College, University of Prince Edward Island, PEI, Canada

Idiosyncratic drug reactions are a rare, but serious complication of drug therapy. Idiosyncratic reactions commonly manifest as fever, skin rash, and hepatic damage, although multiple organs may be involved. Current drug safety assessment programs are unable to successfully identify compounds that may trigger idiosyncratic reactions, largely because they are dependent on the interplay between the chemistry of the compound, environmental factors, and the genetic constitution of the individual experiencing the reaction. Once a drug is in clinical use, we are also unable to accurately identify individuals at risk or experiencing idiosyncratic reactions so that therapy can be modified appropriately. The challenge for pharmacogenomics researchers is to determine if genetic determinants are sufficient and necessary for idiosyncratic drug reactions to occur and if pharmacogenomic profiling can be sufficiently sensitive and specific to be clinically useful in identifying individuals at risk. Further, we must determine if we can use pharmacogenomic information to develop new approaches to identifying compounds likely to be associated with idiosyncratic reactions earlier in the drug development process. This talk will review the state of pharmacogenomics research in idiosyncratic drug reactions.

Alastair Cribb

PEI Health Research Institute and Atlantic Veterinary
College, University of Prince Edward Island,
Charlottetown, Prince Edward Island, Canada

Dr. Alastair Cribb is professor of clinical pharmacology at the Atlantic Veterinary College, UPEI, adjunct faculty in the Department of Pharmacology, Faculty of Medicine, Dalhousie University, and Director of the Laboratory of Comparative Pharmacogenetics at UPEI and Director of the PEI Health Research Institute. In addition, Dr. Cribb is on the Governing Council of the Canadian Institutes of Health Research. Dr. Cribb was previously a CIHR Scholar and is now a Canada Research Chair Holder in comparative pharmacology and toxicology. Dr. Cribb completed his PhD in Clinical Pharmacology at the Hospital for Sick Children, University of Toronto in 1991, after completing his DVM degree at the University of Saskatchewan in 1984. During his PhD, he studied genetic susceptibility to adverse drug reactions. He completed a post-doctoral fellowship in the Faculty of Medicine at Dalhousie University, where he investigated the regulation of cytochrome P450 enzymes during inflammation. He then spent four years in human drug development with Merck & Co, USA where he continued to assess the genetic basis for variable drug response. He joined AVC and UPEI in 1996. Dr. Cribb's current drug and chemical safety research program focuses on molecular mechanisms of drug- and chemical toxicity in humans and animals and the role of genetic variability in determining the response to drug and chemical exposure. He is also investigating the effect of genetic polymorphisms in estrogen formation and disposition on the risk of breast cancer. The CIHR and the Canadian Breast Cancer Research Initiative fund his work

Immune-based Drugs: New Paradigms Challenging Conventional Drug Development Programs.

Antoine A. Noujaim, Altarex, Edmonton, Alberta, Canada

Recent developments in drug discovery have changed the landscape of available pharmaceuticals, thus affecting the costs of both health care delivery systems as well as introducing novel therapeutic modalities armamentarium of prescription drugs. Immune-based biopharmaceuticals are progressively assuming a dominant position of newly introduced agents for the treatment of diverse disease conditions. The challenges faced by both developers of these new agents as well as the regulatory agencies approving them present a dynamic which is constantly changing as more information is gathered on the working of the body's immune system and its response to these new agents. A typical perennial question being: Is an immune response indicative of a clinical response? Conventional drug response curves witnessed when small molecule drugs are tested in humans are often not the case with immune-based drugs. Thus, clinical trial designs, as well as measurable endpoints are often a challenge when developing a biopharmaceutical whose mechanism of action is not totally understood and when pharmacokinetic parameters are practically non-existent. The various aspects regulating the development, manufacturing and testing of such biopharmaceuticals will be discussed in this presentation.

Antoine Noujaim
Altarex, Edmonton, Alberta, Canada

Dr. Noujaim received his B.Sc. in Pharmaceutical Chemistry from Cairo University in 1958. He obtained a M.Sc. and Ph.D. in Bionucleonics as a Fulbright scholar from Purdue University in 1963 and 1965 respectively. Dr. Noujaim joined the Faculty of Pharmacy at the University of Alberta in 1966 as an Assistant Professor and was promoted to Full Professor in 1973. He has chaired the Division of Radiopharmacy for a number of years and was instrumental in the founding of a number of Biotechnology companies in Canada. He was the recipient of many national and international awards including the Old Master, the Distinguished Alumnus of Purdue University, the ASTECH award for technology innovation, Canada's 125 Centennial Award for contributions to the country, and recently an Honorary Doctor of Science from the University of Alberta. Dr. Noujaim has authored and co-authored more than 200 scientific publications.

Bispecific and Bifunctional Nanoprobes: A New Generation of Diagnostics and Therapeutics.

Mavanur R. Suresh, Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

Bispecific and bifunctional antibodies are second-generation monoclonal antibodies incorporating any two desired specificities or functionalities in one molecule. These nannoglues are about the size of natural antibodies or smaller depending on the method of production. Generally, these can be derived from chemical, somatic or genetic engineering methods. The ability of these immunoprobes to act as macromolecular crosslinkers make them ideally suited for a variety of applications in biomedical and biotechnological applications. For example, the bispecific antibodies incorporating two different antigen specificities such as a tumour antigen and a diagnostically useful enzyme marker has led us to develop ultrasensitive immunoassays. The key advantage of these types of probes is that they exhibit the theoretical highest specific activity with every molecule bound to a signal marker for exquisite sensitivities and very clean backgrounds resulting in superior signal to noise ratios. The same probes can be useful in biosensors, biochips and biomems to detect a variety of molecules including cancer antigens, infectious disease markers, environmental contaminants, food microbiology as well as on line real time measurements of various compounds. Another area of application involve the use of these nannoprobes in therapeutics especially drug targeting and pre-targeting. The dual specificity of the molecule allows the pre-accumulation of the probe to the desired disease or target followed by the administration of the therapeutic drug, toxin, radioisotope or even a cytotoxic killer cell.

Mavanur Suresh
Faculty of Pharmacy & Pharmaceutical Sciences,
University of Alberta, Edmonton, Alberta, Canada

Mavanur R Suresh had his early training in India as a botanist/biochemist. His subsequent post-doctoral work was in the area of cancer, tumour antigens and monoclonal antibodies initially at the Scripps Clinic and Research Foundation, La Jolla, U.S.A., and subsequently at the MRC laboratory of Molecular Biology in Cambridge, U.K. He was the Chief Scientist of Biomira Inc, a key Canadian/Alberta biotechnology company for eight years until 1993. He directed the immunodiagnostic program to develop three blood tests against colorectal, breast and ovarian cancer. The breast cancer blood test was the first such FDA approved in the world. The monoclonals that he helped develop and characterize are used in stand alone diagnostic kits as well as some automated formats. He currently holds the CIHR-Industry Chair in Immunoconjugates in the Faculty of Pharmacy, University of Alberta.

SESSION 3

New technologies in drug development

Thomas Spencer, Chief Scientific Officer, Angiotech
Pharmaceuticals, Vancouver, British Columbia,
Canada

Dr. Thomas S. Spencer received his B. S. in Chemistry from the University of North Carolina and his Ph. D. in Physical Chemistry from Colorado State University. He carried out dermatological research at the Letterman Army Institute of Research and later at S. C. Johnson Wax, Inc., in Racine, Wisconsin. During this time, he was a member of the Executive Committee and Program Convenor for the International Society for Bioengineering and the Skin and later served as the President of the society. Subsequently, he was the Vice-President of R&D for Cygnus and afterwards for Pharmetrix, developing transdermal drug delivery systems. At present, he is the Chief Scientific Officer of Angiotech Pharmaceuticals, Inc., responsible for development of drug products for psoriasis, multiple sclerosis, rheumatoid arthritis and vascular restenosis. Dr. Spencer is a member of the Canadian Society of Pharmaceutical Scientists, American Academy of Dermatology, American Chemical Society, American Association of Pharmaceutical Scientists and Society of Cosmetic Chemists.

Water-Soluble Amphiphilic Nanocarriers

Jean-Christophe Leroux, Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University of Montreal, Succ. centre-ville, Montreal (Qc) H3C 3J7, Canada.

Most recently, amphiphilic block copolymers have risen to the forefront of technology pertaining to the preparation of polymeric micelles and sterically stabilized nanoparticles. In the field of drug delivery, particular emphasis has been placed on systems consisting of block copolymers of poly (lactic acid) and poly (ethylene glycol). Our work describes the preparation and characterization of novel micellar systems composed of poly (*N*-vinylpyrrolidone) (PVP) or poly (*N*-2-hydroxypropylmethacrylamide) (PHPMA) and biodegradable polyesters potentially useful for the parenteral administration of water-insoluble drugs. Diblock copolymers are prepared by the ring opening polymerization of the D,L-lactide using semi-telechelic PVP, whereas triblock copolymers are synthesized by free radical polymerisation of HPMA or VP in the presence of a novel biodegradable macromolecular chain transferring agent. These copolymers are characterized by their ability to self-assemble in water at a low critical aggregation concentration (< 10 mg/L) and form soluble colloids of 30-200 nm. Additionally, we herein disclose the synthesis of amphiphilic pH-sensitive diblock and star-shape methacrylate copolymers (unimolecular polymeric micelles) obtained by atom transfer radical polymerization. These polymers are effective for solubilizing hydrophobic drugs, and eventually enabling their oral administration. They are also effective in the formation of stable water-soluble complexes with polyions. Lastly, we describe stabilized, drug-loaded nanodispersions and a method for their production based upon the lyophilization of solvent-water mixtures containing the above-mentioned copolymers.
Labopharm Inc. (Laval, QC), the Natural Sciences and Engineering Research Council of Canada and the Canada Research Chair program, financially supported this work. JCL acknowledges a scholarship from the Fonds de la Recherche en Santé du Québec. This talk was previously given at the International Conference Particles 2002 (April 2002).

Jean-Christophe Leroux
Canada Research Chair in Drug Delivery, Faculty of Pharmacy, University of Montreal, Montreal, Quebec, Canada.

Dr. Leroux is an associate professor at the Faculty of Pharmacy of the University of Montreal. He received his B.Pharm. from the University of Montreal in 1992, followed by a Ph.D. in Pharmaceutical Sciences (1996) from the University of Geneva (Switzerland). From 1996 to 1997, he completed a postdoctoral training at the University of California, San Francisco and then joined the University of Montreal as an assistant professor by the end of 1997. He was promoted associate professor in 2002. Since September 2001, he is the holder of the Canada Research Chair in Drug Delivery. He supervises a group of 15 graduate students and postdoctoral fellows. His research interests include the design of novel biopolymers, stimuli-responsive drug delivery systems (liposomes, micelles and gels) and the targeting of anticancer drugs. He is the author or co-author of more than 40-refereed articles, four book chapters and eight patents/patent applications. He serves in the editorial board of the *European Journal of Pharmaceutics and Biopharmaceutics* and *STP Pharma Sciences.* He has received the Phoenix (1996) and Controlled Release Society-Capsugel (1997) awards for innovative research in pharmaceutical technology. He is a member of the pharmaceutical sciences committee at the Canadian Institutes of Health Research, and serves as a consultant to various drug delivery companies.

Drug delivery systems for paclitaxel: Implantable films and pastes

Helen M. Burt, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada

Paclitaxel is an antimicrotubule agent that binds to microtubules, arrests cells in late G2 or M phases of the cell cycle and is used clinically to treat advanced breast and ovarian cancer. Paclitaxel is also a potent anti-angiogenic agent that inhibits the proliferation of cells such as epithelial cells, vascular smooth muscle cells and fibroblasts. Postsurgical adhesions are abnormal tissue attachments that result from cuts or abrasions to tissues during surgery. The process of adhesion formation involves fibroblast proliferation and angiogenesis. We designed biocompatible, mucoadhesive, implantable polymeric films loaded with paclitaxel that could be applied to the abraded tissues to release the drug over several days and prevent the formation of surgical adhesions. Cross-linked hyaluronic acid (HA) films were prepared containing either free paclitaxel or paclitaxel loaded, low molecular weight poly (L-lactic acid) microspheres. The HA films were characterized by measurements of elasticity, degree of swelling in water and paclitaxel release rates. Paclitaxel was shown to be an effective inhibitor of adhesion formation in a rat cecal side wall abrasion model. Controlled release polymeric paste formulations have been developed in our laboratory for perivascular application to inhibit vascular smooth muscle cell proliferation, inhibit intimal hyperplasia and prevent luminal narrowing after balloon injury in a rat carotid artery model of restenosis. We have also designed injectable polymeric paste formulations of paclitaxel for intratumoral injection, to suppress human prostate tumor growth in mice. The paste formulations were based on a blend of a biodegradable triblock copolymer, poly (D, L-lactide-co-caprolactone)-block-polyethyleneglycol-block-poly (D, L-lactide-co-caprolactone) (PLC-PEG-PLC) with methoxypolyethyleneglycol (MePEG) in a 40:60 ratio. The pastes were characterized using thermal analysis, GPC and paclitaxel release profiles. The controlled release paste formulation could be injected via a 22-gauge needle and was effective in inhibiting LNCaP tumor growth in mice.

Helen Burt
Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada

Dr Burt obtained her B. Pharm. (Hons) in 1975 from the University of Bath, U.K. and her PhD in Pharmaceutics in 1980 from the University of British Columbia, Faculty of Pharmaceutical Sciences. She is currently a Professor in the Faculty of Pharmaceutical Sciences. In November 2001, she was appointed as the CIHR Coordinator in the VP Research Office at UBC. Her research group has been studying the acute inflammatory arthritis caused by the interaction of microcrystals of calcium pyrophoshate and sodium urate with scavenger white blood cells, or neutrophils, in the synovial fluid of joints. This work has been funded by MRC/CIHR Operating Grants since 1982. In the past 8 years, Dr Burt's group has been designing and developing polymer-based drug delivery systems that provide the important features of controlled release of drug and targeted or site-directed delivery to improve drug safety and effectiveness. This work has been funded by an MRC University/Industry grant, CIHR Operating grant, Angiotech Pharmaceuticals Inc. and by the NCE/ Canadian Arthritis Network. Dr Burt has supervised 18 graduate students/post-doctoral fellows and 9 research technicians and has received over $2.0 million in external research funding. She has published over 70 papers in peer-reviewed journals and has several published and filed patents. She was awarded the YWCA Woman of Distinction Award for Science, Research and Technology in 2000, the Angiotech Professorship in Drug Delivery in 1999 and is the recipient of three teaching excellence awards.

The potential use of polymers (Liposomes Or Micelles) as drug delivery systems

Adi Eisenberg, Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Quebec, Canada H3A 2K6

The self-assembly of block copolymers has been the subject of many recent studies. In this talk, a review is presented of the self-assembly phenomenon, notably our ability to control the self-assembly to obtain aggregates of a range of morphologies such as spheres, rods or vesicles. The thermodynamic parameters, which control the morphologies, are the stretching of chains in the core of the aggregate, the repulsion among corona chains, and the interfacial energy. Thus, it is possible to control the morphology by varying the relative block length, polymer concentration in solution, salt content, pH, temperature, etc. Vesicles are among the most promising structures for future consideration in drug delivery, at least if one can extrapolate from experience with the liposomes. In contrast to liposomes, vesicles can easily be prepared under equilibrium conditions. The thermodynamic curvature stabilization mechanism has been elucidated. It consists of preferential segregation of short corona chains to the inside, and long corona chains to the outside of the vesicles. This also allows us to prepare, in one step, vesicles that contain different molecules attached to the inside and the outside surfaces. The unprecedented degree of control and manipulability suggest that block copolymer vesicle is a fertile area for exploration in connection with drug delivery.

Adi Eisenberg
Department of Chemistry, McGill University,
Montreal, Quebec, Canada

After graduation from the Worcester Polytechnic Institute in 1957, Eisenberg received his M.A. from Princeton University in 1959, followed by his Ph.D. in 1960. After a postdoctoral year at Princeton (with A.V. Tobolsky) and another in Basel (with W. Kuhn), he joined the faculty of UCLA in 1962, and moved to McGill in 1967. He was director of Polymer McGill 1991-1998, and became Otto Maass Professor of Chemistry in 1992. Various sabbaticals were spent partly at the Weizman Institute of Science (Israel), Kyoto University (Japan), Imperial College (London), and LURE (Orsay, France), among others. Eisenberg is an author or co-author of over 350 papers in refereed journals as well as over 80 extended abstracts or short articles (in Polymer Preprints, PMSE preprints, encyclopedias, etc.). He co-authored or edited eight books in the field. He has supervised over 40 Ph.D. and M.Sc. students, organized 11 major symposia or international meetings and presents numerous lectures at various universities or companies, as well as invited or plenary lectures at various scientific meetings and workshops. Honors include the Killam Research Fellowship 1987-1989, the CIC (Macromolecular Science and Engineering) Dunlop Lecture award in 1988, the Dow Distinguished Lectureship in 1996, and the EWR Steacie award in Chemistry (CSC) in 1998. Earlier work from Eisenberg's group has centered on ion-containing polymers, which has provided the conceptual framework for understanding architecture - morphology - property relations in random ionomers; his work contributed materially to the increased acceptance of ionomers in a range of commercial applications. He is now involved in the study of the micellization of ionic block copolymers, which show promise as targeted drug delivery vehicles, among others. Aggregates with a very wide range of morphologies have been produced from these materials, including spheres, rods, vesicles, tubules, and a range of unique structures, including some resembling "pincushions". Many of these are morphologically biomimetic. He is co-discoverer of the phenomenon of two-dimensional micellization in block copolymers. Eisenberg is or was a member of the Editorial Advisory Board of the Journal of Polymer Science - Polymer Physics Edition (1977-1999), Macromolecular Reviews (1983-1990), Macromolecules (1986-88) Journal of Non-Crystalline Solids (1989), Polymers for Advanced Technologies (since 1989) and several other publications. He was on the advisory board of the Institute for Amorphous Studies, Michigan and the NSERC Grant selection Committee for Chemistry (1987-1990). He has been a consultant to a number of companies or government laboratories, including the Jet Propulsion Laboratory, Owens Illinois, Energy Conversion Devices, GTE, Raychem, Exxon and Dow Corning, among others. He is a Fellow of the CIC and the APS, and a member of the ACS and the Rheology Society. On all of these, he has held official positions, such as memberships of the Executive Committee or Chairmanships.

Novel Drug Delivery Approaches

William Bosch, Elan Drug Delivery

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.

William Bosch
Director, Drug Dispersion Technology, Elan Drug Delivery, King of Prussia, Pennsylvania, USA

Bill Bosch is Director of Dispersion Technology at Elan Drug Delivery, Inc. He received his Ph.D. in chemistry from the University of Pennsylvania where he studied chemical reaction mechanisms involving metalloporphyrin compounds. Bill was a Visiting Assistant Professor of Chemistry at Colgate University and later a postdoctoral fellow at the University of Zürich in Switzerland. Bill has been involved in the development of NanoCrystal® technology since 1991 when he joined the Sterling Winthrop Pharmaceuticals Research Division, and was a co-founder of NanoSystems LLC (acquired by Elan Corp plc in 1998). His research interests include chemical reaction mechanisms, thermodynamics, X-ray crystallography, and colloid science. Bill is presently responsible for NanoCrystal® dispersion and colloid science research at Elan Drug Delivery, Inc.

SESSION 4

New technologies in drug delivery

Different Aspects of Imaging Techniques and Their Use In Drug Development

Timothy J. McCarthy, Experimental Medicine,
Pharmacia Corporation, St. Louis, MO

In recent years, there has been an increased use of non-invasive imaging techniques in the drug development process. The objective of this presentation will be to illustrate the potential applications of these technologies to both preclincal and clinical drug development. The talk will begin with a brief overview of the major technologies that are available; their limitations in terms of practicality or multi-site feasibility will be highlighted. The remainder of the talk will draw on examples from the literature to illustrate how these types of approaches will help to answer specific questions faced in drug development such as; PK/PD relationships and markers of disease progression or modification.

Timothy McCarthy
Experimental Medicine, Pharmacia Corporation, St.
Louis, Missouri, USA

Dr. McCarthy has a Ph.D. in synthetic organic chemistry from the University of Liverpool, England. In 1991, he joined the research group of Michael J. Welch, Ph.D. at the Mallinckrodt Institute of Radiology, Washington University in St. Louis as a Post-doctoral fellow. During this time, he was involved in the application of organic chemistry to the development of radiopharmaceuticals for Positron Emission Tomography. In 1993, he was appointed to the faculty at Washington University where he continued to develop his interests in all aspects of radiotracer development and evaluation. In 2001, he joined the Pharmacia Corporation where he is currently Group Manager for Tracer Technologies and is involved in the application of non-invasive imaging to drug development. Dr. McCarthy has been an active member of the Society for Non-Invasive Imaging in Drug Development for a number of years and is currently the President-Elect.

PET/SPECT in Evaluation of Novel Dosage Forms

Marc S. Berridge, 3D Imaging

For drug development applications, Nuclear Medicine's imaging techniques are most often applied to measure the functional or biochemical effect of a drug. However, nuclear imaging can also be effectively used to measure the regional distribution and regional pharmacokinetics of a drug or a dosage form. Imagine the ability to do regional biopsies throughout the whole human body, and then extract and quantify the drug throughout each organ, and do so every few seconds or few minutes as you choose. Regional pharmacokinetics in the organs of interest enriches traditional pharmacokinetic data that represents only the blood. While detailed data can be a benefit during the development of many types of drugs, it is of critical importance in certain specialized applications. Positron Emission Tomography (PET) and Single Photon Emission Tomography (SPECT) have just begun to be seriously explored as tools for development of new drugs. This presentation explores the work that has been done to date to apply PET and SPECT to regional biodistribution measurements of drugs, and dosage forms. Examples show the effect of changes in formulation on regional biodistribution and kinetics. The areas most strongly represented so far are the inhaled drug formulations. These are orally inhaled formulations for pulmonary drug delivery, and nasally inhaled formulations for delivery of drug to the nasal mucosa, turbinates, and sinuse. The technology has also been used to investigate the performance of delayed release and intestinal-release oral dosage forms, and for other regional or topical drug applications. The unique information that is supplied by the use of *in-vivo* drug imaging correlates well with conventional pharmacokinetic evaluations. It can enhance, explain or predict pharmacokinetic or even clinical trial results. Knowledge of drug distribution can be used to take appropriate action during drug development. Used effectively, imaging data can prevent good drugs from being rejected for the wrong reasons, and can provide earlier detection of drugs that are not performing and need to be rejected.

Marc Berridge
Department of Chemistry, Case Western Reserve University, Cleveland, Ohio, USA

Marc Berridge received the BS degree at Carnegie-Mellon University (Chemistry), and MS and PhD degrees at Washington University in St. Louis (Organic radiochemistry, with concentration in radiopharmaceuticals for positron tomography). He has been involved in development and production of positron-emitting radiopharmaceuticals for research and clinical use, and in the use of PET scanning of animals, of human volunteers and of human patients for research and clinical purposes. Much of his more recent research has been for drug development, involving the investigation of quantitative regional distribution and pharmacokinetics of inhaled drugs in human subjects. His work has taken place at the French Atomic Energy Commission (CEA) Orsay, France, at the University of Texas Medical Center at Houston, and for the last eleven years at Case Western Reserve University in Cleveland. He has recently joined 3D Imaging, a contract research organization dedicated to application of radiopharmaceuticals and positron tomography to drug development.

Biomarker Applications Of Positron Emission Tomography

Daniel E. Salazar, Clinical Discovery, Bristol-Myers Squibb Company, Princeton, New Jersey, United States of America

Pipeline pressure and the increasing costs of drug development are driving the search for biomarker technologies that will support efficient evaluation of lead compounds. Positron emission tomography (PET) imaging studies can assess *in vivo* drug pharmacokinetics, target occupancy and a wide range of pharmacodynamic effects. It is conceivable that eventually PET imaging surrogate endpoints will emerge. This suggests that PET imaging may be increasingly employed in drug development. This presentation will provide a brief summary, with examples from the literature and the authors' experience, on the use of PET imaging in drug development. The pros and cons of PET imaging as a biomarker technology platform from an industry perspective will be discussed.

Daniel Salazar
Director, Clinical Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey, USA

Dr Salazar completed his undergraduate education at Princeton University where he majored in Biology. He spent the next 6 years conducting research in the cellular and molecular aspects of endogenous resistance to cancer. He continued his formal education at the Department of Pharmaceutics, SUNYAB. His doctoral research focused on elucidating the metabolic and pharmacokinetic mechanisms of increased drug induced toxicity in obesity. Upon completion of his doctoral requirements, Dr Salazar continued to work at SUNYAB, contributing to the development of pharmacokinetic-pharmacodynamic models of corticosteroid effects on leukocyte circulation. Dr Salazar has spent the last 13 years in the pharmaceutical industry first within the Clinical Pharmacology Department at Marion Laboratories in Kansas City, MO and more recently in the Clinical Discovery at Bristol-Myers Squibb Co, Princeton NJ. During this time, he has directed over 70 clinical Phase I/II studies investigating dosage form bioequivalence, drug absorption, first administration to man, pharmacokinetics, pharmacodynamics, disease state interactions and drug-drug interactions. Dr Salazar is the coauthor of over 35 articles in peer-reviewed journals and in 1995 was named to the editorial board of the Journal of Clinical Pharmacology. Dr Salazar received the McKeen Cattell Memorial Achievement Award of the American College of Clinical Pharmacology in 1998. In 1999, Dr Salazar was elected to the Board of Directors of the Society for Nuclear Imaging in Drug Development and in 2001 was named a Fellow of the American College of Clinical Pharmacology.

Merck Company Foundation Undergraduate Summer Student Award Recipients and Poster Competition.

Kishor M. Wasan, National Director, Faculty of Pharmaceutical Sciences, University of British Columbia

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(s) Title of Project

Evan H. Kwong, 3rd Year Pharmacy
  Dr. Thomas K. H. Chang, Dr. Marc Levine, Pharmacogenetics of Codeine Metabolism to Morphine in Pediatric Dental Patients: A Pilot Study

Genevieve Faucher, 3rd Year Pharmacy
  Dr. Jean-Christophe Leroux, Micelles polymeres pour la chimiotherapie due cancer.

Melissa Skanes, 1st Year Pharmacy
  Dr. Vernon Richardson, Anti-inflammatory activity of a Tripterigium triterpene in an in vitro model: Regulation of cytokine induction/matrix, metalloproteases expression.

Jennifer Bonnetta, 2nd Year Pharmacy
  Dr. F.J. Burczynski, The Role of Intracellular binding proteins on the hepatic availability of cellular substrates

Rudy C. Sedlak, 2nd Year Pharmacy
  Dr. James Diakur, Dr. Leonard I. Wiebe, Galactose-Armed Cyclodextrins for Targeted Drug Delivery

Marc Grenier, 3rd Year Pharmacy
  Dr. Robert Drobitch, Potential Role of Ginseng Induced Nitric Oxide production in modulating Cytochrome P450 medicated metabolism

Annie K.Y. Cheung, 3rd Year Pharmacy
  Dr. Micheline Piquette-Miller, Cytokine Mediated Pathways of MultiDrug Resistance Gene Regulation.

Ruth Ombati, lst Year Pharmacy
  Dr. Adil Nazarali, Effect of antiepileptics on palatogenesis and the role of the Hoxa2 gene.

Phillippe Vincent, 2nd Year Pharmacy
  Dr. Pierre M. Belange, The Identification and Characterization of a Novel Hydroxylated Metabolite of Diclofenac (Voltaren)

Kishor Wasan
National Director of Canadian Undergraduate Pharmacy Student Research Program, Associate Professor & Chair of Pharmaceutics, University of British Columbia, Vancouver, British Columbia, 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 years that Dr. Wasan has been an independent researcher at UBC, he has published over 70 peer-review articles and 110 abstracts in the area of lipid-based drug delivery and lipoprotein-hydrophobic drug interactions. 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 Grant in Pharmaceutics and Pharmaceutics Technologies. In January 2001, with funding from the Merck Company Foundation, Dr. Wasan established a Canadian Summer Student Research Program for Undergraduate Pharmacy Students. 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.

SESSION 5

Biotech drug development challenges

Iain J. McGilveray
McGilveray Pharmacon, Ottawa, Ontario, Canada

Dr. McGilveray obtained a Ph. D in Pharmaceutical Science in Glasgow, Scotland, trained in pharmacokinetics, with Prof. Riegelman at the U. of California, San Francisco, and worked in drug research in Health Canada for over 30 years. His research interests have included, biopharmaceutics, bioanalytical methodology, dissolution, pharmacokinetics, metabolites, pharmacodynamics and chiral effects, but principally have been focused on bioavailability and bioequivalence and he has contributed to more than 150 research publications. Dr McGilveray is a Fellow of the AAPS and Canadian Institute of Chemistry. In addition, he is past-chair (1999) of the Regulatory Sciences section of AAPS. He has organized and participated in many scientific conferences internationally, most recently for the AAPS 2002 Toronto meeting. He has participated in several DIA workshops and received the Distinguished Career Award in 1998. He is interested in new scientific approaches to drug regulation and harmonization of standards, having been advisor to WHO and FDA. He is a consultant, adjunct professor (medicine) University of Ottawa and was chair of the CSPS June 2001 symposium in Ottawa

Gordon McKay
PharmaLytics Inc., Saskatoon, Saskatchewan, Canada

Dr. Gordon McKay received his bachelor's degree in Biochemistry from the University of Saskatchewan and went on to complete his Ph.D. in 1981 with Peter Shargool specializing in enzymology. 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 started in the College of Pharmacy with support from the MRC in the form of a Program Grant. The research program is aimed at investigating ways to improve the efficacy of psychotropic 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 has been a faculty member in the College of Pharmacy and Nutrition since 1989 and is currently a full professor of Pharmacy on leave of absence in order to establish a new research institute called Pharmalytics Inc. in the research park. At present, he is chief executive officer of this corporation but continues to teach in the College of Pharmacy and Nutrition.

Proteomics

John J. M. Bergeron, Chair, Department of Anatomy and Cell Biology, Faculty of Medicine, McGill University, Montreal, Quebec; Director, Montreal Proteomics Center, Genome Quebec, Canada

The promise of proteomics is to characterize all proteins of all cells in normal and disease conditions. Organelle isolation from cells overcomes the major hurdle in current proteomics efforts by enabling an extension of the dynamic range of protein expression in the cell. Several thousand proteins in organelles of the secretory pathway have been characterized by this approach with the unexpected observation that several hundred 'novel' proteins have been uncovered. The assignment of these 'novel' proteins to specific organelles enables a rapid elucidation of their function. In this way, virtual organelles are now being solved leading to the ultimate resolution of the virtual cell.

John Bergeron
Faculty of Medicine, McGill University, Montreal, Quebec, Canada

Dr. John J.M. Bergeron is Professor and Chair of the Department of Anatomy and Cell Biology at the Faculty of Medicine of McGill University. He received his D. Phil. from Oxford University as Rhodes Scholar. He worked as a Postdoctoral Fellow in Rockefeller University and as Scientific Staff at National Institute for Medical Research in London before joining McGill University. Dr. Bergeron is also the Director of Genome Quebec/Genome Canada Montreal Proteome Center. Dr. Bergeron has published more than 185-refereed articles and has been an Invited Speaker to numerous conferences and symposiums, and has trained over 25 Graduate Students and more than 20 Postdoctoral Fellows. He was elected the Fellow of the Royal Society of Canada in 1995. He has served as a committee member for the National Cancer Institute of Canada, Canadian Institute of Health Research, Fonds de la Recherché en Sante du Quebec, US Public Health Service, International Human Frontier Science Program, U.S. Army Medical Research for Breast Cancer Research Program and is on the Editorial Board of several journals. Dr. Bergeron is also Chief Scientific Officer for Proteomics Division of Caprion Pharmaceuticals, a biotech company applying organellar proteomics to the acceleration of drug discovery to diagnose and treat prominent diseases.

Vaccine Formulation, Delivery and Stability

Carl C. Burke, Vaccine Pharmaceutical Research
Department, Merck Research Laboratories, Merck &
Co., Inc., West Point, PA 19486 USA

Vaccine pharmaceutical research involves the use of scientific, pharmaceutical, and engineering principles to design and develop generally well-tolerated, immunogenic, stable, and scaleable vaccine dosage forms. Some of the topics to be covered in this presentation include physicochemical properties of vaccine antigens (macromolecules and microorganisms), types of adjuvants and delivery systems used to potentiate vaccine-induced immune responses *in vivo*, and causes of vaccine inactivation during storage. Selected case studies will be used to illustrate various formulation approaches used to ensure maintenance of vaccine potency during preparation, storage, and administration.

Carl J. Burke
Director, Vaccine Pharmaceutical Research
Department, Merck Research Laboratories, Merck &
Co., Inc., West Point, Pennsylvania, USA

Dr. Burke is a Director in the department of Vaccine Pharmaceutical Research at Merck Research Laboratories. He joined Merck in 1990 after obtaining a Ph.D. in Biophysical Chemistry at Temple University. He has worked on formulation and characterization of proteins and vaccines as well as process development and transfer to manufacturing. His recent efforts have been focused on development of stabilizing formulations and processes (e.g., lyophilization) for live viral vaccines.

Inhalation Delivery of Protein Drugs

Lynn Van Campen, Inhale Therapeutic Systems, Inc.

Drug delivery via inhalation is a mix of old and new technologies. The delivery of drug therapeutics to the lung to treat respiratory conditions, such as asthma via nebulization and the more convenient metered dose inhaler (MDI), dates back to the 1950's. By 1990 however, there was a growing realization that the CFC propellants used in MDIs contributed to the depletion of the ozone layer, and that their replacement would require much time and resources from the pharmaceutical industry. Interest turned to the invention and development of new inhaler technologies that avoided the need for CFCs, such as dry powder delivery and new modes of nebulizing from portable devices. Drug delivery via inhalation has seen a renaissance in the past decade, and new start-up companies have proliferated to realize its fresh potential. An important aspect of new developments in the field has included the concept of delivering protein therapeutics to the deep lung with an efficiency of systemic uptake that could compete with injection. Mid- to late-stage clinical studies of peptides and proteins delivered as dry powders or as nebulized solutions are demonstrating clinical success in growing patient populations. The dramatic impact on healthcare of these new inhalation systems used to deliver drugs across many therapeutic classes could be only a few short years away. Examples of these development programs will be reviewed, with a special look at the new formulation and device strategies being developed to address the challenges of handling and delivering proteins via the lung.

Lynn Van Campen
Inhale Therapeutic Systems, Inc., San Carlos,
California, USA

Lynn Van Campen joined Inhale Therapeutic Systems, Inc. in San Carlos, CA in 1996 as Vice President of Pharmaceutical Development. Inhale is known first for its dry powder inhalation technology, directed primarily toward the non-invasive delivery of proteins and peptides to the lung as a route to systemic delivery. Prior to joining Inhale Dr. Van Campen was Director of Pharmaceutics at Boehringer Ingelheim Pharmaceuticals, where she was responsible for the development and clinical manufacture of MDIs, DPIs, inhalation solutions and other dosage forms. During this time she served as a member of the PhRMA Pharmaceutical Development Steering Committee, as well as a member of the Scientific Advisory Panel for the IPAC prior to departing Boehringer. Prior to obtaining her Ph.D. in Pharmaceutics at the University of Wisconsin, she was employed by Pfizer, Inc. where she first became familiar with inhalation dosage form technology.

Mass Spectrometry - The panacea or not?

Alfred Yergey, National Institute of Child Health and Human Development, Maryland

Mass spectrometry is a central technology in the development of modern pharmaceuticals. As a quantitative tool in determining drug absorption, distribution and the characterization of metabolites, mass spectrometry is unsurpassed, for the qualitative analysis of small molecules, it is also remarkably powerful. In other areas of pharmaceutical development, total dependence on this technology is unwise at best. For example, mass spectrometry has a role in the systematic investigation of potential drug targets, but it is only one of the armouries of tools available. Techniques such as two dimensional gel eletrophoresis, immunoassays and several other evolving tools such as gene and protein arrays, have a critical role. Dependence on mass spectrometry alone would be akin to performing a trio sonata on a solo instrument: a bravura performance that lacked depth and missed the full range of the music's beauty. At present, no single technology can be considered dominant for the task of identifying drug targets because each provides only a single voice of the final complex score. Understanding the strengths and weaknesses of existing technologies is central to their adroit application. In this talk, the features that have placed mass spectrometry in its unique position in the pharmaceutical industry will be discussed. Importantly, the increasing trend towards higher molecular weight drug development (e.g., recombinant proteins, antibodies, peptides, and other biopolymers), places different demands on the technique. The rapidly evolving area of proteomics, while based around mass spectrometry at this stage, will increasingly demand alternative analytical strategies. The capital investment required for mass spectrometry, its relative complexity and throughput rate are issues. The absence of commercial sources for large scale 2D gels, and the difficulties with generating high purity antigens required for reliable antibodies are just a few of the limitations of the available analytical options. Appropriate parallel use of all of these technologies must be considered in the light of the challenges posed by directed proteomics. We are facing new and remarkably demanding tasks when we consider the requirement to identify and quantify hundreds if not thousands of proteins in a single sample and over a dynamic range of 8 to 10 orders of magnitude. These specific challenges and some of the potential solutions will be discussed in detail.

Alfred Yergey
National Institute of Child Health and Human Development, Bethesda, Maryland, USA

Alfred Yergey, B.S. in Chemistry, Magna cum laude - 1963, Muhlenberg College; PhD in Chemistry (Physical) - 1967 under the direction of F.W. Lampe. He was a postdoctoral fellow at Rice University with J.L. Franklin, 1967-69. After 2 years at Esso Research and Engineering, he joined Scientific Research Instruments in Baltimore where he worked with a group led by M.L. Vestal in designing and producing the first commercial CI sources. He came to NIH in 1977 where he was active in the quantification and human metabolic kinetics of small endogenous molecules using LC/MS, glucose, cortisol and acetylcholine. In addition, he was heavily involved in developing mass spectrometric, clinical and mathematical tools for the study of whole body calcium metabolism. He is currently a Section Chief in the NICHD. Dr. Yergey's current research interests lie in the areas of 1) equilibrium ion-molecule chemistry applied to hydration thermodynamics and 2) problems of protein characterization with emphasis on de novo sequencing and protein-protein interactions. Dr. Yergey is a member of the American Society for Mass Spectrometry, the American Chemical Society, the Canadian Mass Spectrometry Society, the Association of Biomolecular Resource Facilities (ABRF) and the American Assoc. for the Advancement of Science.

Comparability and Other Regulatory Issues for Biotech Drugs

Anthony Ridgway, Manager, Biotherapeutics Division, Biologics & Radiopharmaceuticals Evaluation Centre, Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada

Demonstration of biocomparability is important when significant changes are introduced into a manufacturing process for a biological drug and, if successful, may relieve the manufacturer of the need to repeat preclinical or clinical studies. It is heavily influenced by the ability to manufacture proteins to high levels of purity and the capability of modern analytical techniques to thoroughly characterize them. There are also circumstances where clinical testing may be desirable despite the weight of analytical data suggesting comparability. The Canadian regulatory perspective will be presented along with some consideration of relevant ICH guidance on biotechnology products. In addition, other regulatory issues particular to new technologies and new types of biotechnology-derived drugs will be discussed.

Anthony Ridgway
Manager, Biotherapeutics Division, Biologics & Radiopharmaceuticals Evaluation Centre, Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada

Dr. Anthony Ridgway was born and educated in the U.K. and obtained his B.Sc. and Ph.D. in biology from McGill University, Montréal. From 1982-85 he conducted post-doctoral research at the Cancer Research Laboratory (CRL), University of Western Ontario, London, Canada, and continued as Assistant Professor, CRL and Dept. of Microbiology and Immunology, from 1985-91. From 1989-91 he held a National Health AIDS Research Scholar award. Academic research activities included work on the structure and expression of oncogenes, retroviral regulatory elements, HIV regulatory genes, and inducible expression vectors. In 1991, Dr. Ridgway joined Health Canada to help regulate biological drugs derived via biotechnology. He is currently Manager of the Biotherapeutics Division, Biologics & Radiopharmaceuticals Evaluation Centre (BREC), whose responsibilities include product testing, bioassay research and regulation of biopharmaceuticals including cell and gene therapies, hormones, cytokines and monoclonal antibodies. He has been active with ICH* since 1993, serving on Expert Working Groups addressing the quality of biotechnology products. In June 2000, he was elected to the US Pharmacopoeia Committee of Experts on Gene Therapy, Cell Therapy and Tissue Engineering. (*International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use)

Challenges in developing a biotech drug product

Leanne Embree, Angiotech Pharmaceuticals, Inc., Vancouver, British Columbia, Canada

The development pathway for drugs and biopharmaceuticals offers many challenges. Often challenges are overlooked or underestimated; yet, the potential for successfully marketing a product clearly necessitates overcoming these challenges. The regulatory dimension is added to the significant challenges of the particular science and technology needed to develop a drug product suitable for marketing. In addition, development of a new therapeutic drug product is a slow process that requires years of research and clinical trials. It is estimated that 12 to 15 years are spent developing a new drug. Up to 6.5 years are anticipated for the early discovery and pre-clinical testing with an added 7 years of clinical testing, followed by 1.5 years for regulatory review of the application to market the product. Biopharmaceutical development may take even longer due to complicated technologies and indications, plus their novelty to the regulatory agencies. The major phases of product development following the discovery phase are product formulation, pre-clinical efficacy & toxicity testing, intellectual property protection, clinical assessment, process development with scale-up, and regulatory review. Each phase presents its own challenges. The cost of developing a pharmaceutical product is estimated to be around $800M, when failed drug efforts are included in the total costs. With return on investment based on a projected market date, the time spent in development must be used wisely and all time delays must be kept to a minimum during product development. More precisely a one-month delay in development for a potential $240M product is $20M lost forever or $666,000 per day. Insight into the product-specific challenges early in the development phase is critical to managing time and funding. Potential areas for significant challenges in the development of a pharmaceutical product will be presented along with some ideas and procedures on how to address these challenges.

Leanne Embree
Analytical Chemistry, Angiotech Pharmaceuticals, Inc., Vancouver, British Columbia, Canada

Dr. Embree is Director of Analytical Chemistry in the Research and Development Department of Angiotech Pharmaceuticals, Inc. She received her Ph.D. (1989) in pharmaceutical chemistry from the University of British Columbia, Vancouver, Canada. Following post-doctoral studies with the MRC Regulatory Peptide Group in the Faculty of Medicine at the University of British Columbia, she joined the Investigational Drug Program in Medical Oncology at the British Columbia Cancer Agency in 1990. As part of the Investigational Drug Program, she implemented quality systems to enable drug development from bench-top through to Phase II clinical testing. In addition, her research activities included clinical therapeutic drug monitoring for busulfan, which is used as preparative chemotherapy prior to bone marrow transplantation and development of analytical methods for quantitation of platinum antineoplastic agents in blood. Dr. Embree joined Angiotech Pharmaceuticals, Inc. in 1997 and presently holds the title of Director. She actively participates in Research and Development activities, has corporate responsibilities for analytical chemistry and is currently implementing a quality management system. In addition, Dr. Embree is a member of CSPS, RAPS, DIA, AAPS and ACS and actively serves the profession as reviewer for various analytical journals and as member of the PSG West Coast Course Committee for the Pharmaceutical Sciences Group.

POSTER PRESENTATIONS

New technologies in drug discovery & drug development

Posters numbered 1-25 will be on display in MB252 from 8-5 Thursday.
Posters numbered 26-50 will be on display in MB252 from 8-5 Friday.

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

1. CELLULAR UPTAKE OF DNA FRAYED WIRES.

***Robert Macgregor*, Department Of Pharmaceutical Sciences, Faculty Of Pharmacy, University Of Toronto, 19 Russell Street, Toronto, Ontario.**

Purpose. We are investigating the cellular uptake of novel multistranded DNA structures called Frayed Wires (FW). DNA Frayed Wires arise from self-association of oligonucleotides with long 3'- or 5'-terminal runs of consecutive guanines, e.g. $d(A_{15}G_{15})$. The aggregated structures consist of a guanine stem and single-stranded non-guanine arms. The "parent" oligonucleotides aggregate to form a polydisperse mixture of species that can consist of up to 15 monomers, or more. FW exhibit unusual thermal and enzymatic stability relative to other DNA structures such as duplex DNA and the G-quartet. **Methods**. In order to examine possible biological applications of this structure, we have compared the uptake of radiolabel led DNA FW in HepG2 and CNE1 cell lines to that of normal single-stranded DNA. **Results**. Cellular uptake of FW was greater than that of single-stranded DNA by both HepG2 and CNE1 cells with a greater difference observed in HepG2 cells. This uptake process exhibited characteristics similar to other active transport processes such as saturation, temperature dependence, substrate concentration dependence, and inhibition by metabolic inhibitors. Uptake of FW was considerably reduced at 4°C but remained higher than that of single-stranded DNA. The initial rate of FW uptake was concentration dependent and maximal above 0.3 μM. Uptake of both FW and single-stranded DNA was reduced by heparin but the uptake of FW was inhibited largely. FW derived from other parent strands, namely $d(T_{15}G_{15})$, $d(G_{15}A_{15})$, and $d(N_{15}G_{15})$ demonstrated similar cellular uptake patterns as those from $d(A_{15}G_{15})$, indicating the importance of the guanine stem rather than the arms in determining cellular uptake. Electrophoretic structural analysis of extracellular and internalized FW indicated that they were not metabolized and maintained their structures after uptake. **Conclusion**. DNA FW is taken up via an energy dependent process. Their uptake is more rapid than that of single stranded DNA. At least fractions of the FW that enter the cells maintain their multistranded structure.

2. CHARACTERIZATION OF ATENOLOL DOSE - PR-INTERVAL PROLONGATION IN THE RAT.

***John Clements* and Fakhreddin Jamali; Faculty of Pharmacy & Pharmaceutical Sciences, University Of Alberta, Edmonton, Alberta, Canada.**

Purpose: It has been shown that an interferon-α2a induced inflammatory state can reduce the electrocardiographic response of cardiac â-adrenergic receptors to a β-blocking drug. Knowing that hydroxylmethylglutaryl coenzyme-A reductase inhibitors (statins) are now recognized as having anti-inflammatory properties, we hypothesize that pravastatin may attenuate the ability of the inflammatory state to inhibit normal cardiac response to the â-blocking drug atenolol. The purpose of this study was to find an atenolol dose that will allow for the detection of subtle changes in cardiac response as measured by PR-interval prolongation and heart rate. **Methods:** Metal electrodes were implanted into the right and left axilla of male Sprague-Dawley rats (weight ranged from 239-320gm) the day prior to the experiment. Over night, the rats were fasted for 12 hours, but had free access to water. The following morning, after obtaining baseline ECGs, the rats received single doses of 1.25, 2.5, 5.0 or 10 mg/kg (n=5-7/-group) atenolol in solution by oral gavages. The PR interval and heart rate was monitored by ECG for the next 6 hours. Food was introduced at 3 hours post-atenolol dose. **Results**: There was a significant prolongation of the PR interval following all doses of atenolol. The responses were, however, highly variable. After all doses, the effect increased with time until reaching a maximal value in 2.3 to 2.7 h. There was a dose-response relationship (mean±SEM maximum PR interval prolongation of 6.1±1.8, 7.3±2.3, 11.2±2.9, 13.5±2.8% for 1.25, 2.5, 5.0 and 10 mg/kg doses, respectively). It appears that the effect of atenolol on PR interval increases with the elevation of dose up to 5-10 mg/kg when a plateau is reached. **Conclusion**: Doses of 2-5 mg/kg appear to be suitable for pharmacodynamic studies. The variability of the response, however, must be addressed in future studies. Supported by the CIHR.

3. EFFECT OF GINSENG EXTRACT G115 ON ETHOXYRESORUFIN O-DEETHYLASE ACTIVITY IN GLIAL CELL CULTURE: TIME AND CONCENTRATION DEPENDENCE AND ROLE OF NITRIC OXIDE.

Ping Sun, *Robert Drobitch*, College Of Pharmacy, Faculty Of Health Professions, Dalhousie University, Halifax, N.S.

Objective: To evaluate the effects of ginseng extract G115 on cytochrome P4501A1/2-mediated ethoxyresorufin O-deethylase (EROD) activity in glial cell cultures, to determine the time and concentration dependence on EROD activity and the role of nitric oxide in modulating EROD activity. **Methods:** Ginseng G115 was obtained from Pharmaton. Cells were isolated from the brains of newborn (<24hr) Sprague-Dawley rats and cultured in 20% FBS and Dulbecco's MEM until confluent (approximately 1 week). Cultures contained approximately 95% astrocytes and 5% microglia. Cells were exposed to G115 preparations (or media) + dibenzanthracene in serum-free media for 24hrs at concentrations ranging from 0.005 to 2.0 mg/ml. At 24hr, aliquots were removed for measurement of lactate dehydrogenase (LDH) release (as a measure of cell viability) and nitrite levels (as a measure of NO production). Remaining media was then removed and replaced with fresh serum free media containing ethoxyresorufin and returned to incubation conditions. Resorufin concentrations were determined fluorometrically after a 3-hr incubation period. Finally, cell plates were scraped and homogenized for determination of protein and total LDH present in cell cultures. For time course studies, cells were exposed to G115 at 0.1mg/ml and DBA for up to 48 hrs. At specific time points, levels of nitrite were determined, and ginseng-containing media removed and EROD activity determined as above. The effects of 1400W (a specific inhibitor of nitric oxide synthase) on ginseng-induced responses was also examined. The cells were exposed for 24 hours with 1400W alone prior to the addition of ginseng at either 0.1 or 0.5 mg/mL and incubated for an additional 24 hrs prior to the determination of EROD activity and nitrite levels. **Results:** G115 produced a concentration-dependent decrease in EROD activity at 0.05 and 0.1 mg/mL, with an increase in EROD activity at concentrations ranging from 0.05 to 0.75 mg/ml with a maximum occurring at 0.25 mg/mL. In time-course studies, G115 at 0.1 mg/mL inhibited EROD activity at 6hrs of incubation, with levels returning to control levels at 12 hrs and significantly increasing at time points thereafter. Nitrite levels in culture increased significantly above control at all time points studied. **Conclusion:** Ginseng has the capacity to modulate astrocyte EROD activity, which may have implications for drug interactions in this organ. The inductive effect is likely not modulated by nitric oxide. **Acknowledgements:** Funding for this project was provided by an establishment funds from the Dalhousie University College of Pharmacy and Faculty of Health Professions, and the Dalhousie University College of Pharmacy Pharmacy Endowment Fund.

4. HEMODYNAMIC AND NEUROHORMONE EFFECTS OF DILTIAZEM IN RATS.

Pollen Yeung, **J.D.Z. Feng, D. Fice, and A. Jarvis; Pharmacokinetics and Metabolism Laboratory, College Of Pharmacy & Departments Of Medicine (Cardiology), Dalhousie University & Qeii Health Sciences Centre, Halifax, N.S., Canada**

Introduction Hemodynamic and neurohormone effects are critical factors responsible for safety and efficacy of many cardiac drugs. Despite its clinical importance in cardiac drug therapy, regulation of hemodynamic parameters by neurohormone mechanisms is far from understood. Design and Methods An exercise rat model (male SD rats weighing about 500 g) was used to study the hemodynamic and neurohormone effects. Prior to the study, a polyethylene catheter was implanted into the right carotid artery of each rat under general anaesthesia for hemodynamic recording and blood sample collection. After recovery (24 h), a blood sample (0.5 mL) was taken from each rat for measurement of plasma catecholamines. One group will receive DTZ (s.c. 10 mg/kg bid for 5 doses), and the other normal saline. Each rat will be exercised on a treadmill (7 m/min and 3% slope) for 7 min. BP and HR measurement were recorded throughout the exercise and up to 1 h after the exercise. Blood samples were obtained at 0 (just before exercise), 5 and 7 min during exercise, and at 5, 30, and 60 min post exercise. Plasma concentrations of catecholamines were determined by HPLC. Results Exercise increased SBP from 107 to 123 mmHg (15%), HR from 428 to 473 bpm (11%), and plasma norepinephrine concentrations from 0.34 to 0.81 ng/mL in control rats. In DTZ treated rats, SBP increased from 96 to 104 mmHg (8.3%), HR from 391 to 441 (13%), and norepinephrine from 1.9 to 2.7 ng/mL. Conclusion DTZ attenuated the hemodynamic and neurohormone response to exercise in normotensive SD rats. Acknowledgement Supported in part by a Research Support Grant from Dalhousie Pharmacy Endowment Foundation

5. KIDNEY SIDE EFFECTS OF COX2 INHIBITORS ARE NOT COX1/COX2 RATIO RELATED.

Sam Harirforoosh **and Fakhreddin Jamali; Faculty of Pharmacy and Pharmaceutical Sciences, University Of Alberta, Edmonton, Alberta, Canada.**

Introduction: Cyclooxygenases (COX) are key enzymes forming arachidonic acid to prostaglandins. COX1 and COX2 are two different isoformes of cyclooxygenase. Nonsteroidal anti-inflammatory drugs (NSAIDs) have different selectivity to inhibit COX1 and COX2. Treatment with NSAIDs has been associated with kidney side effects in human, and animals. Impaired glumerular filtration rate (GFR), a decreased in urinary sodium and potassium excretion, and papillary necrosis are common renal dysfunctions of NSAIDs. **Objective:** The objective of the research was to examine the possible correlation between renal effect and COX2 inhibition of various NSAIDs with different COX1/COX2 ratio inhibition in the rats. Therefore, we have examined the effect of rofecoxib, 30 mg/kg; celecoxib, 120 mg/kg; etodolac, 370 mg/kg; meloxicam, 9 mg/kg; diclofenac 30 mg/kg; and flurbiprofen 125 mg/kg on renal function of rats. **Methods:** Each medication was administered orally once a day for four days. Control group received methylcellulose as vehicle. Renal function was assessed by measurement of urine volume and urinary sodium and potassium excretion. Urinary sodium and potassium excretion was studied using a gas analyzer. Urine was collected for eight hours after dosing. The changes of urine volume and sodium excretion between two groups were compared using t-test. **Results:** Treatment with Rofecoxib, celecoxib, etodolac, diclofenac and flurbiprofen decreased sodium and potassium excretion significantly. The sodium excretion values were 1.05 rofecoxib vs. 1.48 control, $P< 0.002$; 0.76 celecoxib vs. 1.64 control, $P<0.0001$; etodolac 0.44 vs.1.75 control, $P<0.0001$; diclofenac 0.26 vs. 1.68 control, $P< 0.0001$; and flurbiprofen 0.41 vs. 1.75 control, $P<0.0001$. Potassium excretion values were 2.05 rofecoxib vs. 2.46 control, $P< 0.032$; 1.66 celecoxib vs. 2.43 control, $P<0.002$; etodolac 1.31 vs. 2.32 control, $P<0.0001$; diclofenac 0.94 vs. 2.42 control, $P< 0.0001$; and flurbiprofen 1.34 vs. 2.32 control, $P<0.0001$. In contrast, treatment with meloxicam did not change sodium or potassium retention significantly. **Conclusion:** Since the kidney side effects of NSAIDs are not COX1/COX2 ratio related, the extent of distribution into kidney becomes a plausible explanation. Supported by the Canadian Arthritis Network (NCE).

6. STEROID WEANING & TACROLIMUS DOSING IN PEDIATRIC HEART TREANSPLANT PATIENTS ARE RELATED TO THE MDR1 POLYMORPHISMS AT EXONS 21 & 26.

Hongxia Zheng, S. Webber, A. Zeevi, E. Schuetz, J. Zhang, J. Lamba, P. Bowman1, G. Boyle, Y. Law, S. Miller, F. Pigula, G.J. Burckart, Schools of Pharmacy & Medicine, University of Pittsburgh, Pittsburgh, PA; St. Jude Children's Research Hospital, Memphis, TN, USA.

P-glycoprotein (P-gp), a transmembrane product of the multidrug resistance gene (MDR1), acts as an energy-dependent efflux pump expelling its substrates out of cells. In transplant patients, P-gp is critically involved with T-cell apoptosis and the pharmacokinetics of corticosteroids, cyclosporine, tacrolimus (Tac), and sirolimus. Various polymorphisms of MDR1 gene have been identified, and a silent mutation in exon 26 and a G-T mutation in exon 21 have been correlated with P-gp expression and function in humans. Purpose: The objective of this study was to evaluate whether the MDR1 exon 26 and 21 polymorphisms are associated with Tac disposition and steroid weaning in pediatric heart transplant (HTx) patients. Methods: Seventy-seven pediatric HTx patients were studied. Tac blood concentration (ng/ml) per dose (mg/kg/day) at 3 and 12 months post HTx was calculated. Attempts were made to wean all patients from steroids as clinically indicated, and the prednisone dose at 12 months was recorded. MDR1 genotyping was determined by PCR amplification, sequencing the DNA, and sequence evaluation using Polyphred software (University of Washington) to identify the CC, CT and TT variants of exon 26 and the GG, GT, and TT variants of exon 21. Results: For the Tac level/ dose in the pediatric HTx patients, the exon 26 data revealed that the CC patients were lower than the CT/TT patients were (mean 36.4 vs. 65.4, $p<0.05$ at 3 months; 42.4 vs. 72.9, $p=0.05$ at 12 months). For exon 21, the GG patients were lower than the GT/TT patients were (mean 40.3 vs. 68.7, $p<0.05$ at 3 months; 38.7 vs. 78.6, $p<0.05$ at 12 months). For steroid weaning at 12 months post HTx for exon 26, 12 of 18 (67%) patients in the CC group were still on prednisone, whereas only 19 of 48 (40%) of the CT/TT group were still receiving prednisone ($p=0.04$). For exon 21, 12 of 19 (63%) patients in the GG group were still on prednisone in comparison to 17 of 46 patients (40%) of the GT/TT group ($p=0.06$). Conclusion: Pediatric HTx patients who are homozygous for the C allele or the G allele for MDR1 exon 26 and 21 mutations respectively are significantly more likely to continue to require prednisone at 12 months. These CC and GG patients have a significantly lower Tac blood concentration per dose compared with CT/GT/TT patients. The linkage between exon 26 and 21 occurred because 15 of 16 CC patients were also GG. Taken together, the heterozygous/TT patients with lower P-gp pump function might have better Tac and prednisone absorption, and get an accentuated pharmacological response to immunosuppressants that allows them to be weaned from steroids more readily than the homozygous patients. Whether this is simply due to changes in drug absorption or is due to alterations of P-gp and apoptosis in T cells is unclear, but confirms the importance of P-gp function and genotype in organ transplant patients.

7. SYN 1193, A NEW FLUOROQUINOLONE, AS AN ALTERNATIVE TO VANCOMYCIN IN THE RABBIT MODEL OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) PERITONITIS IN PERITONEAL DIALYSIS.

Sharon Mitchell, F. Jamali, M. Larabie, M. Dasgupta

Background: With the development of Vancomycin Resistant Enterococci (VRE) and vancomycin intermediate resistant *Staphylococcus aureus* (VISA), it is essential that the use of vancomycin be limited and new alternatives developed to prevent further vancomycin resistance. Staphylococcal infections are a common cause of peritonitis in patients receiving Peritoneal Dialysis (PD). Intraperitoneal (i.p.) vancomycin has been the empiric treatment of choice for these infections. In this study, SYN 1193, an investigational fluoroquinolone, was studied as an alternative to vancomycin in an established rabbit model of peritonitis in PD, infected with a virulent strain of MRSA (MU 7056). **Hypothesis:** 1. Intraperitoneal (i.p.) SYN 1193 would be effective in treatment of MRSA peritonitis in PD in the rabbit model compared to i.p. vancomycin and controls. 2) That the pharmacokinetic/pharmacodynamic (PK/PD) profile of i.p. SYN 1193 in plasma and PDF would be appropriate for the treatment of peritonitis as determined by Area Under the Curve/ Minimum Inhibitory Concentration (AUC/MIC) >100 and a Cmax/MIC >8. **Methods**: Twelve SPF New Zealand White rabbits (3 groups of 4) were studied. Silastic PD catheters were implanted in the peritoneal cavity and tunneled subcutaneously to exit over the lumbar spine(Day 1). Peritonitis was induced by inoculation of catheter exit sites with MRSA (MU7056)(Days 4,6&7). Following confirmation of infection by PDF bacterial culture, the rabbits were dialyzed 4 times a day. The test drug was given i.p in the dialysate twice daily (bid) Days 11-15 – Group1 Control (no drug), Group 2 (SYN1193 20 mg/kg bid), Group 3 (vancomycin 20 mg/ kg bid). A complete set of plasma, PDF and urine samples were taken on the first and fourth days of treatment to determine the PK/PD parameters of ke, ka, t1/2, Cmax, tmax, Cmax/MIC, AUC and AUC/MIC. The rabbits were sacrificed on Day 15. Efficacy was determined by bacterial culture and identification of PDF between study groups. **Results:** 1) Eleven of twelve rabbits were successfully infected. One rabbit (vancomycin group), chewed the catheter, became very ill and was euthanised. Pre-treatment colony counts (mean± Standard Error (SE)) per group were Control $1.65\text{x}10^6 \pm 1.28\text{x}10^3$, SYN 1193 $4.33 \text{ x } 10^5 \pm 2.28\text{x}10^5$ and vancomycin $4.99 \text{ x } 10^4 \pm 3.91\text{x}10^4$. Control rabbits, very ill without treatment, were sacrificed Day 11. Day 15, PDF in 3 of 4 rabbits in the SYN 1193 group and 3 of 3 in the vancomycin group showed no growth. Day 15, log reductions of colony counts (mean±SE) of $5.12 \text{ x } 10^{-1} \pm 5.26 \text{ x}10^{-1}$ were achieved with SYN1193 and $3.96 \pm 5.92\text{x}10^{-1}$ with vancomycin. This was not statistically significant, p=0.23 (unpaired t test, 2 tailed) indicating similar efficacy of SYN 1193 and vancomycin. Pharmacodynamic studies of SYN 1193 showed that 20mg/kg bid ip achieved a Cmax/MIC ratio of 689 in plasma and 6,935 in PDF, and AUC/MIC ratio of 1487 in plasma and 24843 in PDF, which is more than adequate for treatment of this infection. **Conclusions:** SYN 1193 i.p., in lieu of vancomycin, may be appropriate for further studies towards the treatment of MRSA infections, in humans with CAPD peritonitis. NSERC and Synphar are gratefully acknowledged for their support.

8. AMPHOTERICIN B INTERACTIONS WITH A DOPC MONOLAYER, ELECTROCHEMICAL INVESTIGATIONS.

Robin Stoodley, **K. Wasan, D. Bizzotto; Department Of Chemistry, University Of British Columbia, Vancouver, BC, Canada; Faculty Of Pharmaceutical Sciences, University Of British Columbia, Vancouver, BC, Canada**

Purpose: Amphotericin B's influence on a lipid monolayer was studied using novel electrochemical methods in an effort to characterize Amphotericin B - lipid interactions at the molecular level and to examine proposed mechanisms of action. **Methods:** A model lipid membrane consisting of a monolayer of dioleoyl phosphatidylcholine (DOPC) adsorbed onto a mercury electrode has been used to study the interaction between the lipid and different formulations of Amphotericin B (Fungizone, Heat-treated Fungizone, and Abelcet). **Results:** The organizational order of the lipid on the electrode undergoes two phase-changes passing through three distinct organizational states as applied electric potential is varied. The lipid organizational order was measured by electrochemical methods (capacitance and metal ion (Tl+) reduction) with and without Amphotericin B, characterizing the change in lipid order due to interaction with the drug. The mean size and number density of pores in the monolayer were estimated by fitting the reduction current transients to a random array of microelectrodes model. This method was shown to be sensitive for investigation of the interaction of drugs with a pure lipid monolayer. **Conclusions:** Abelcet was found to have a smaller disruptive effect on lipid order than Fungizone and Heat-treated Fungizone. The formulation used to solubilize the Amphotericin B was also studied; Sodium deoxycholate used as a solubilizer in Fungizone displayed significant influence on lipid order similar to that observed for Abelcet. The lipid complex that is used in Abelcet did not significantly influence the DOPC monolayer order. The lipid present in the Abelcet may have an annealing or healing effect that buffers the disruption possible due to Amphotericin B.

9. A PHYSIOLOGICALLY RELEVANT LIPID BILAYER MODEL FOR STUDYING TRANSCELLULAR PERMEATION VIA MOLECULAR DYNAMICS.

Eric Carpenter, **D. Ridgway, Y. K. Tam, J. Tuszynski.; Department of Physics, University of Alberta, Edmonton, AB; Kinetana Group Inc., Edmonton, AB**

Purpose. There is substantial interest in techniques for early determination of ADME properties, including in silico prediction of absorption. Advances in molecular mechanics and computational modeling have made it possible to directly simulate certain aspects of absorption on a molecular scale. Our purpose is to develop a molecular model of a physiologically relevant lipid bilayer adequate for use in simulation studies of transcellular permeation. **Methods**. We typically construct bilayers consisting of 32 DPPC lipid and 12 to 32 cholesterol molecules in a 32 Å x 32 Å x 60 Å box with periodic boundary conditions, for a surface area per head group of 64 $Å^2$. Simulations are performed at constant NVT using the MM3 force field and the Tinker molecular dynamics package. Molecules are oriented in a bilayer with initially random nonoverlapping transverse positions, in an enlarged periodic cell, which is then shrunk while performing energy minimizations. Water is then added around the outside of the layer to the boundary of the box, for approximately 14000 atoms in the simulation. Finally, the system is time stepped to equilibration at 310 K (37°C). The simulations are monitored by internal energy, lateral pressure, and visualization of trajectory frames. **Results and Discussion**. To be useful in permeation studies the model membranes should consist of a mix of appropriate molecules, be numerically stable, equilibrated into an appropriate phase, and be at physiologically reasonable temperature and pressure. Additionally, it must be large enough to accommodate the drug, and achieve time scales sufficient to capture effects of interest. DPPC is a common membrane lipid and is the most widely studied, however studies using pure DPPC may not be physiological, as cholesterol substantially stiffens the lipid tails. Our selected concentrations are representative of the 30% to 50% molar seen in mammalian cells. Randomization of initial conditions ensures that the membrane anneals to a disordered transverse state. Measurements of lateral forces (surface tension) within the membrane show strong dependence on head group surface area, consistent with physiology and the picture of a relatively incompressible two-dimensional liquid. Internal energy equilibrates within 10 ps, and runs of 100 ps are both stable and achievable, allowing simulations involving gauche/trans isomerization and thus realignments of lipid tails, but not flipping, rotations, or large-scale displacement of entire lipid molecules. The volume simulated is such that a single drug molecule in the box is at a concentration of 50 mM. **Conclusion**. By construction, this bilayer is physiologically reasonable; large enough to avoid finite volume effects, and achieves timescales sufficient to model the most important lipid behaviours during permeation. Since the timescales of drug permeation are not achievable, we instead plan to study the energy barrier posed by the membrane through simulations with the drug molecule constrained to pass through the membrane, and relate this barrier to in vitro permeability's and in vivo absorption. We hope that this physically and physiologically motivated approach will improve on ad hoc in silico models and lead to increased insight into the process of drug absorption on a molecular scale.

10. COMPARATIVE EFFECT OF VALPROIC ACID, A-FLUORINATED-VALPROIC ACID, AND CAPRYLIC ACID ON PLASMA 8-ISOPROSTANE LEVELS IN RATS.

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Purpose: Recently, we demonstrated in rats an association between valproic acid (VPA) administration and an increase in plasma 8-isoprostane levels, which is used as an *in vivo* indicator of oxidative stress. This increase by VPA is enhanced by phenobarbital pretreatment, which is known to induce cytochrome P450-mediated VPA biotransformation, but is not decreased by SKF-525A or 1-aminobenzotriazole, which inhibits VPA biotransformation. To gain insights into the mechanism by which VPA increases oxidative stress, the objective of this study was to compare the influence of structurally similar, 8-carbon fatty acids such as VPA, α-F-VPA, and caprylic acid (octanoic acid) on the production of 8-isoprostane. **Methods**: Adult male Sprague-Dawley rats (250-350 g, N = six per treatment group) received single ip doses of saline (vehicle control), VPA (500 mg/kg), α-F-VPA (500 mg/kg), or caprylic acid (500 mg/kg). At 0.5 h post administration, blood samples were obtained and plasma 8-isoprostane levels were determined by EIA kit (Cayman Chemical Co.). In a positive control experiment, rats were treated with carbon tetrachloride (2 mL/kg, ip) or corn oil (vehicle control) and blood samples were obtained 4 h later. **Results**: As expected, carbon tetrachloride increased (7-fold) plasma 8-isoprostane levels when compared with the control group. By comparison, VPA produced a 3.5-fold increase in levels of 8-isoprostane (124 ± 8 pg/mL) compared to saline control (35 ± 3 pg/mL), whereas α-F-VPA also elevated 8-isoprostane (91 ± 11 pg/mL), but the levels were significantly lower than the VPA-treated group. In contrast, caprylic acid had no effect. **Conclusions**: Our novel findings indicate that not all structurally similar, 8-carbon; linear fatty acids produce oxidative stress because VPA, but not caprylic acid, increases plasma 8-isoprostane levels. The α-F-VPA-mediated increase in 8-isoprostane levels is not likely a consequence of β-oxidation because this compound is not subject to fatty acid β-oxidation. **Acknowledgements**: This research was supported by the Canadian Institutes of Health Research (Grant MOP-13744 to F.S.A. and T.K.H.C.). V.T. received a Pharmacy Graduate Scholarship and T.K.H.C. a Research Career Award in the Health Sciences from the Canadian Institutes of Health Research and the Rx&D Health Research Foundation.

11. CYCLOSPORINE EXPOSURE USING A FAST/FOOD TEST MAY BE A SENSITIVE TOOL FOR IDENTIFYING ABSORPTION PROFILE DIFFERENCES BETWEEN CYCLOSPORIN FORMULATIONS.

Paul Keown **(Canada), A Johnston (USA)**

Purpose: Cyclosporine (CsA) absorption is a complex process that contributes to pharmacokinetic variability and may be a factor affecting clinical outcomes in transplantation such as graft survival. Although Neoral absorption has been extensively tested, generic CsA products may exhibit differences in absorption characteristics. The goal of this analysis was to use the CsA Exposure Fast/Food Test (CEFFT) to identify possible differences in the CsA absorption profile between Neoral (published data) and Eon (available in the public domain) (cyclosporin modified USP). **Methods**: Analysis was performed on data from healthy volunteers in two separate studies which provided CsA AUC, Cmax and Tmax values (PK) following single doses of Neoral (n=24) or Eon (n=18). Subjects in both studies were randomized to receive the CsA oral formulation either with a high-fat meal (Food) or under fasting conditions (Fast) and PK profiles completed. After a 14-day washout period, each subject received CsA alternatively with Fast or Food condition. Fast/Food ratios for PK parameters for each CsA formulation were compared. **Results**: Mean Fast and Food AUC values (± s.d.) for Neoral were 3514 (878) and 2981 (865) ng.h/mL; and for Eon were 3925 (728) and 4443 (991) ng.h/mL. The Fast/Food ratios for Neoral and Eon were 1.18 versus 0.89 respectively demonstrating a fundamental difference in the absorption profiles between the two formulations. CsA absorption from Neoral is greater during the fasted state versus the fed state, but the opposite effect occurred with CsA absorption from the Eon formulation. A similar trend was observed with Cmax Fast/Food ratios (Neoral = 1.33 versus Eon = 0.91). The Fast/Food ratio for Eon is closer to the ratio for Sandimmun (0.74), which is not bioequivalent to Neoral. **Conclusions**: CEFFT may be a sensitive test for comparing CsA absorption characteristics between different formulations. CEFFT identified different absorption characteristics between Eon and Neoral and the CsA exposure differences may be up to 30% between the two formulations. CsA absorption from Eon appears to be closer to Sandimmun than Neoral based on CEFFT testing.

12. IN VITRO TRANSPORT AND UPTAKE OF MATRINE BY THE CACO-2 CELLS.

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Purpose. The goals of this work are: (a) to characterize the transepithelial transport and cell uptake of matrine in the Caco-2 cells. Matrine is a major alkaloid of a Chinese anticancer herb, *Sophora subprostrata*, and (b) to investigate whether P-glycoprotein modulates the intestinal absorption of matrine. **Methods.** Caco-2 cells were purchased from the American Type Culture Collection (Manassas, Va) and grown in Dulbecco's modified Eagle's medium at 37^0C in a humidified atmosphere of 5% $C0_2$ and 95% air. When the cells were between passage 55 and 75, they were seeded as a monolayer of 0.5×10^6 cells in the inserts of a 12-well plate for 23-27 days and cultured to confluency. Before the uptake study, the culture medium was replaced with the Hanks balanced salt solution (HBSS) and matrine was applied from either the apical or the basolateral side of the cell monolayer. At pre-determined time points, aliquots (150 µl, apical → basolateral transport; 40 µl, basolateral → apical transport) of HBSS were taken from the opposite chamber compartment and replaced by equal volumes of fresh HBSS. The amount of permeated matrine in the HBSS samples was determined by capillary electrophoresis equipped with an ultraviolet detector fixed at 214 nm wavelength. Capillary electrophoretic separation was conducted under a 25 kV voltage in isopropanol/Tris-phosphate buffer at pH 5.5. **Results.** Capillary electrophoretic analysis showed only unchanged matrine in the HBSS samples. No matrine metabolite was detected. The influence of time on the flux of matrine across the Caco-2 cell monolayer was determined at initial matrine concentrations of 3.2, 1.6, and 0.8 mM. The time course was linear at all matrine concentrations for ~0.5 h in both directions of the Caco-2 cell monolayer. When the incubation temperature of the cells was decreased from 37^0C to 25^0C, a decrease of transport rate was observed. The presence of saturability and the sensitivity to temperature suggested the involvement of an active process in the cellular uptake of matrine. The active process probably operated in the basolateral to apical direction with an average P_{app} of $167.13 \pm 123.15 \times 10^{-6}$ cm/s, which was about 11 times higher than the apical to basolateral flux (P_{app} $14.33 \pm 4.98 \times 10^{-6}$ cm/s). When matrine was co-administered with 50 µM of verapamil (a P-glycoprotein inhibitor), the basolateral to apical flux was decreased by about 50%. Similar results were observed when the monolayer was pre-incubated with verapamil from the apical side before the addition of matrine. **Conclusion.** Results of our studies indicate that matrine transport occurs *via* an active process and is bi-directional. Moreover, matrine is a substrate of P-glycoprotein in the gastrointestinal tract.

13. METABOLISM AND DEGRADATION OF MOMETASONE FUROATE IN RAT & HUMAN BIOLOGICAL FLUIDS & TISSUES.

Xiaowei Teng, **Cheung, H.T.A., Cutler, D.J., Davies, N.M.*; Faculty Of Pharmacy, University Of Sydney, NSW 2006, Australia**

Mometasone furoate[#] (MF) is a synthetic glucocorticoid used in the topical treatment of dermatoses and rhinitis, and has recently been introduced in the treatment of asthma. The drug has high local potency at the application site while presenting relatively low systemic potency. The systemic availability after oral administration is approximately 1%. The reason for this is probably a combination of high affinity to the glucocorticoid receptor and rapid biotransformation in the liver. **Purpose:** The objective of this study is to characterize the kinetics of *in vitro* and *ex vivo* metabolism of MF in rat and human. **Methods:** The *in vitro* metabolism of MF was evaluated in biological fluids and tissues of human and rat. The biological matrices used include human plasma, rat serum, human and rat urine, and microsomal or S-9 fractions of tissues of human and rat liver, intestine and lung. The MF remaining, and the degradation and metabolic products formed were measured by reversed phase HPLC with UV detection. Chemical structures of the degradation and metabolic products were elucidated using data from mass spectrometry, and ^{1}H and ^{13}C NMR and UV spectroscopy. The kinetic profiles were assessed using SCIENTIST™. **Results:** Qualitative and quantitative differences in transformation profiles of mometasone furoate were observed among biological media of both human and rat. Degradation was the major event in human plasma, rat serum, and human and rat urine. 9β,11β-epoxidation (**A**) and 17 side-chain dehydration and re-arrangement (**B** and **C**) were major degradation routes in human plasma and rat serum, while in both human and rat urine, 9β,11β-epoxidation (**A**) was a major event and 17 side-chain dehydration and re-arrangement are minor routes (**B** and **C**). Metabolism of mometasone furoate occurred primarily and rapidly in microsomal fraction of both human and rat liver, appreciably in microsomal fraction of human intestine, mitochondrial and nuclear fractions of rat liver, and everted rat intestine, but minimally in cytosolic fraction of rat liver, S-9 fractions of rat lung, stomach, spleen and kidney, and human lung. Under the experimental conditions, 6β-hydroxy-mometasone furoate was the major metabolite formed in rat liver S-9 and microsomal fractions, and everted rat intestine. Preliminary investigation using chemical inhibitors revealed that CYP3A was the major CYP 450 enzyme responsible for metabolism of mometasone furoate. **Conclusion:** The rat appears to be a suitable model for metabolism of MF as it qualitatively responds in a similar degradation and metabolic profile to humans. *Present address: College of Pharmacy, Washington State University, Pullman, WA. Schering-Plough Australia Pty Ltd. kindly supplied #Mometasone furoate.

14. STRUCTURAL CHARACTERIZATION OF MAJOR DEGRADATION & METABOLIC PRODUCTS OF MOMETASONE FUROATE.

Xiaowei Teng, **Cheung, H.T.A., Davies, N.M.*, Cutler, D.J., Tattam, B.N.; Faculty Of Pharmacy, University Of Sydney, NSW 2006, Australia**

The new synthetic and potent anti-inflammatory drug mometasone furoate[#] (MF) was found to decompose into four major degradation products **A**, **B**, **C** and **D** in aqueous solutions in a pH-dependent manner, [1] and in biological fluids and tissues. In addition, the drug was found to be biotransformed primarily to a metabolite **M** in rat liver *in vitro* and everted rat intestine *ex vivo*.[2] **Purpose:** The objective of this study was to isolate, characterize and determine the structure of the four decomposition products and the metabolite. **Methods:** The products were isolated and purified by preparative HPLC using a Beckman ultrasphere octyl column (150 mm, 10 mm I.D., 5 μm) with a mobile phase of methanol: water (6:4, v/v), monitored by a photodiode array detector. The isolated and purified products were analysed by mass spectrometry, ^{1}H and ^{13}C NMR and UV spectroscopy. **Results:** From the UV, mass spectral and ^{1}H and ^{13}C NMR data, it is demonstrated that MF degrades to product **A** with loss of hydrogen chloride and the formation of an 9β,11β-epoxidation. Subsequent loss of water from **A** from the side-chain forms **C**. In parallel, mometasone furoate itself decomposes into product **B** with a similar loss of water, followed by subsequent loss of hydrogen chloride to yield product **C**. Hydrolysis of the furoate ester group of product **A** gives rise to product **D**. The metabolic transformation of mometasone furoate consists of 6β-hydroxylation to give **M**. **Conclusion:** The four degradation products **A** ($C_{27}H_{29}ClO_6$), **B** ($C_{27}H_{28}Cl_2O_5$), **C** ($C_{27}H_{27}ClO_5$), and **D** ($C_{22}H_{27}ClO_4$) are respectively 21-chloro-17α-hydroxy-16α-methyl-9β,11β-oxidopregna-1,4-diene-3,20-dione 17-(2-furoate), 9α,21β-dichloro-11β,21α-dihydroxy-16α-methylpregna-1,4,17,20-tetraen-3-one 21-(2-furoate), 21β-chloro-21α-hydroxy-16α-methyl-9β,11β-oxidopregna-1,4,17,20-tetraen-3-one 21-(2-furoate), and 21-chloro-17α-hydroxy-16α-methyl-9β, 11β-oxidopregna-1, 4-diene-3, 20-dione. The major metabolite **M** ($C_{27}H_{30}Cl_2O_7$) is 9α, 21-dichloro-6β, 11β, 17α-trihydroxy-16α-methylpregna-1, 4-diene-3, 20-dione 17-(2-furoate). Present address: College of Pharmacy, Washington State University, Pullman, WA. [#]Mometasone furoate was kindly supplied by Schering-Plough Australia Pty Ltd. [1] Teng, X.W., Davies, N.M., Cutler, D.J. Degradation of Mometasone Furoate: Influence of pH, Ionic Strength, and Simulated Biological Fluids. *Proceedings of the Canadian Society of Pharmaceutical Sciences 4th Annual Symposium,* Ottawa, Canada June 2001, In: *Journal of Pharmacy & Pharmaceutical Sciences* 2001: 4(2): 8 P118. [2] Teng, X.W., Davies, N.M., Cutler, D.J., Brown, K.F. Clinical and Pre-Clinical Degradation and Metabolism of Mometasone Furoate: Rat versus Human. *Proceedings of the Canadian Society of Pharmaceutical Sciences (CSPS) 3rd Annual Symposium* Vancouver, Canada June 2000 A 25-1.

15. PHARMACOKINETICS OF HALOFANTRINE ENANTIOMERS AFTER INTRAVENOUS ADMINISTRATION TO HYPERLIPIDEMIC RATs.

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Purpose. Halofantrine (HF) is a highly lipophilic antimalarial compound that is known to bind to plasma lipoproteins. In this study the pharmacokinetics of HF enantiomers were studied in rats rendered hyperlipidemic by administration of poloxamer 407. **Methods.** Two days prior to study, male Sprague Dawley rats (n=4) were administered poloxamer 407 (1g/kg) or saline (n=6 rats) by ip injection. The day before study, cannulae were implanted into the right jugular vein. Rats were given 2 mg/kg of HF HCl intravenously on the study day, followed by collection of blood samples for 48 h. Plasma samples were assayed using stereospecific assay. Plasma lipid levels were also determined in a satellite group of control and poloxamer 407-treated animals. Plasma protein binding was determined using an erythrocyte binding method. **Results.** The following HF pharmacokinetic results were obtained (mean±SD): Poloxamer pretreatment caused substantial reduction (~ 10-fold) in CL and Vdss of both HF enantiomers. Although stereoselectivity was retained for CL, after poloxamer 407 the stereoselectivity in Vdss was diminished. Total plasma cholesterol and triglyceride levels were 8- and 33-fold higher in poloxamer rats, respectively, compared to control rats. Mean plasma unbound fractions for the (+) - and (-)-HF, respectively, were 9.1 and 8.1-fold higher in normolipidemic than in hyperlipidemic plasma. **Conclusions.** The pharmacokinetics of HF is strongly influenced by hyperlipidemia, presumably because of increased binding to plasma lipoproteins. Funding from Burroughs Wellcome/AFPE (AACP-NIP grant; DRB) and Canadian IHR (KMW).

	CL, mL/h/kg		Vdss, L/kg		t½, h	
	(+)	(-)	(+)	(-)	(+)	(-)
Control	171±20[a]	270±46	3.33±0.76[a]	5.12±1.30	17.1±6.7	10.1±1.52
Poloxamer	15.7±4.5[a,b]	23.0±7.0[b]	0.13±0.06[b]	0.14±0.09[b]	18.7±8.2	8.86±1.49[b]

a. Significantly different from (-) enantiomer. b. Significantly different from control rats.

16. PLASMA CORTICOSTERONE AND STRESS RESPONSE AFTER CANNULATION SURGERY: APPLICATION OF A NOVEL CORTICOSTERONE HPLC METHOD.

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Purpose. Activation of the hypothalamic-pituitary-adrenal (HPA) axis by physiological stressors such as chronic cannulation and experimental stress leads to increased circulating levels of cortisol in humans and corticosterone in rats. As stress may influence pharmacokinetics and pharmacodynamics of drugs, as well as subsequent stress responses, it is necessary to determine baseline levels of corticosterone during recovery of the HPA-axis from jugular vein cannulation. HPLC methods for corticosterone analysis have been developed that are specific and sensitive. They, however, require long extraction times with large volumes of solvent. We report the development of a reversed-phase HPLC method with a simple extraction method for the determination of corticosterone in rat plasma. We examined baseline corticosterone levels in rats following jugular vein catheterization and their subsequent response to restraint stress. **Methods.** Rat plasma was extracted with 5.0 ml ethyl acetate, washed with 0.1 *M* sodium hydroxide and then with water. The extract was analyzed by HPLC on a C_{18} column with ultraviolet absorbance detection at 254 nm. Pooled rat plasma was treated with activated decolorizing carbon to remove endogenous corticosterone, and was then used to prepare standards for the assay. Male, Sprague-Dawley rats (n=4) were cannulated in the right jugular vein, and then blood sampled for 7 consecutive days for the determination of plasma corticosterone. On the 7th day, rats were exposed to restraint stress prior to blood sampling. **Results.** Using 0.5 ml plasma for extraction, the detection limit of the assay was 10 ng/ml, and the recovery of corticosterone after extraction ranged from 84 to 87%. The standard curve was linear over the concentration range 10-500 ng/ml. The coefficient of variation for intra-day and inter-day precision ranged from 1.0 to 6.9% and 0.8 to 10.0%, respectively, and accuracy was within 8% of the spiked standards over the concentration range. After jugular vein cannulation, plasma corticosterone levels were elevated, and then declined progressively until it reached the baseline level over the next 6 days. On the 7th day of recovery, corticosterone levels were elevated in response to restraint stress. **Conclusion.** The method involves a simple extraction method for the determination of corticosterone in rat plasma. Jugular cannulation causes increased corticosterone concentration, but does not inhibit subsequent response to restraint stress. This work was supported by the Canadian Arthritis Network (NCE).

17. RHEUMATOID ARTHRITIS (RA) REDUCES BIOTRANSFORMATION OF LOSARTAN TO ITS ACTIVE METABOLITE.

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Purpose: Losartan (LOS) is an anti-hypertensive agent of angiotensin II receptor antagonist class. The main pharmacological activity of LOS is attributed to its metabolite EXP-3174 (EXP) formed upon the first-pass through the liver. Inflammatory conditions may affect metabolism. We tested our hypothesis that increased expression of inflammatory mediators reduces formation of EXP. **Methods**: Three groups of normotensive subjects were included (I: 14 with active RA, II: 12 with controlled RA (remission) and III: 7 healthy volunteers. Their arthritis index was measured and physical assessment was made. They were not on any drug known to interact with LOS. They were dosed orally with 100 mg LOS tablets with 240 ml water. Blood samples were collected at 0, 15, 30, 45 min, 1, 2, 3, 4, 5, 8 and 12 h post-dose and analyzed for LOS and EXP. Plasma C-reactive protein (CRP), tissue necrosis factor (alpha) (TNF) and NO were measured at time zero. AUC ratio of EXP over LOS (EXP/LOS) was considered as the extent of metabolism of the drug. **Results:** Significantly greater concentrations of pro-inflammatory mediators were found in one or other RA group as compared with the healthy group. RA reduced EXP AUC in both groups. Significant linear relationships were found between arthritis index and various variables including , NO and EXP/LOS. In addition, CRP concentration significantly correlated with NO. **Conclusion**: Biotransformation of losartan to its active metabolite was reduced by RA due, likely, to a reduced hepatic clearance caused by pro-inflammatory mediators. The therapeutic significance of this finding remained to be tested.

Subjects	CRP	TNF	NO	EXP AUC	EXP/LOS
Healthy	9.0±7.7	<2	11.1±5.6	4.2±2.0*	3.8±2.6
Active	30.1±29.1*	3.7 ± 1.0	30.7±15.2*	1.7±0.6	2.3±1.5
Remission	8.7±8.0	18.1±17.3*	16.7±7.5	2.4±1.1	2.9±1.9

**Significantly different from other groups (ANOVA/Duncan p=0.05)*

18. SUPPRESSION OF HEPATIC AND INTESTINAL P-GLYCOPROTEIN AND CYP3A IN INFLAMMATION.

***Julie Kalitsky-Szirtes*, M. Piquette-Miller; University Of Toronto, Toronto, Canada**

Purpose. Inter-individual differences in hepatic and intestinal expression of cytochrome P450 3A (CYP3A) and P-glycoprotein are important contributors to variations in drug bioavailability. Endotoxin-induced inflammation is known to affect the expression of numerous liver-derived proteins and enzymes. Suppression of hepatic levels of P-glycoprotein/*mdr1* in inflammation has recently been shown. As drug efflux and metabolism in the intestine also poses a barrier to drug absorption, we examined the influence of endotoxin-induced inflammation on the intestinal and hepatic expression of CYP3A, *mdr1a*, *mdr1b* and *mrp2* in rats. **Methods.** Male Sprague Dawley rats (250-300g; n=6) were administered endotoxin (LPS, 5mg/kg) or saline *ip* and tissues collected at 12-24 h. Levels of mRNA were measured by quantitative RT-PCR in samples isolated from whole liver or intestinal enterocytes (duodenum, jejunum, ileum or colon). GAPDH normalized optical density ratios of mRNA levels are reported as percentage controls. **Results.** As compared to controls, significantly lower *mdr1a* mRNA levels in liver (29 ± 9.8% controls) and intestine (51 ± 9.8 % controls) were observed in the LPS-treated rats. Similar reductions in *mdr1a* mRNA were seen in all four intestinal segments whereas *mdr1b* mRNA levels were not significantly altered in treated rats. LPS-treated rats displayed significantly lower levels of CYP3A mRNA in both liver (48 ± 5.5% controls) and jejunum (30 ± 8.4% controls). Lower levels of *mrp2* mRNA were also observed in jejunum samples from treated rats (49 ± 4.7% controls). Functional transport studies with PGP and CYP3A substrates in Ussing Chambers are currently underway, and will be presented. **Conclusion.** Expression of *mdr/mrp* drug efflux transporters and CYP3A drug metabolizing enzymes are suppressed during inflammation. As changes in expression of these proteins are found in both intestine and liver, an increased bioavailability and altered clearance of P-glycoprotein/ CYP3A substrates may result. As inflammation is a component of many diseases, these findings highlight a potential source of inter-individual variability or drug-disease interactions, and may be an important consideration in bioavailability studies. Grants: CIHR/Rx&D. Abstract presented in part at AAPS Annual Meeting, Oct 21-25, 2001. AAPS PharmSci Supplement 3(3):R5273.

19. AN INTEGRATED STRATEGY FOR DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS IN SUPPORT OF PRECLINICAL DRUG DISCOVERY STUDIES.

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During the preclinical development of a new drug candidate, a diverse number of analytical studies relating to chemistry, formulation, animal pharmacology, toxicology, manufacturing and clinical supplies are required to meet the regulatory requirements of an Investigational New Drug (IND) submission. At the onset of a preclinical program, careful planning of an integrated strategy for all analytical chemistry requirements is paramount, and doing so will result in significant savings in time and resources. This review poster provides a brief overview of the choices of analytical methods such as GC, HPLC, GC/MS and LC/MS techniques commonly encountered in a preclinical program and their applications in the analysis of bulk pharmaceutical materials, formulation intermediates and finished products are discussed. The development and validation of an analytical method supporting a number of integrated preclinical studies applications are also discussed in the context of regulatory requirements. Strategies in the design of analytical methods in support of drug measurements in biological samples commonly encountered during preclinical animal and toxicology studies are presented, including the need for integrating the requirements for method cross-validation between biological sample matrices. This discussion offers a potential model for biotech and pharmaceutical discovery companies during out-sourcing of analytical studies.

20. APPLICATION OF A MINIBORE COLUMN FOR THE HPLC ANALYSIS OF FLAVONOIDS IN ST JOHN'S WORT PRODUCTS.

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Purpose: Most complementary medicines contain a multitude of chemical components, some of which are claimed to contribute to the biological activity of such products. Quality control of these products is an important requirement to ensure that high quality products are made available to the consumer. The objective of this study was therefore to develop a suitable HPLC method to assay the various components in St John's Wort dosage forms for quality control purposes, using a narrow bore column. **Methods:** A gradient HPLC method using a Luna 5µm C_{18} (2) 150 x 2.00mm minibore HPLC column and UV detection, was developed for the separation of rutin, isoquercitrin, hyperoside, quercitrin, quercetin and kaempferol. The development process involved a systematic investigation of gradient conditions, flow rate, and temperature. **Results:** A gradient system according to Table 1 below was developed.

Solvent A: acentonitrile
Solvent B: 0.5% phosphoric acid
(pH = 1.5)

Time (mins)	% A	% B
0	14.5	85.5
10	14.5	85.5
12	40	60
25	30	70

Table 1. Gradient for analysis of flavonoids in St John's Wort products.

This method was subsequently applied to assay selected commercially available St John's Wort products. **Conclusion:** The application of a minibore (2.0 mm i.d) column for the HPLC assay of components in St John's Wort has several advantages over the more commonly used wider bore (4.6 mm i.d) columns. This system provided the necessary accuracy, precision and reproducibility and was associated with several advantages when compared to using standard bore (4.6 mm) HPLC columns. The low flow rates (200-300 µl/minute) result in a significant decrease in amounts of solvent used with a consequent decrease in cost. The minibore column provides a potential gain in efficiency together with high-resolution capability and associated improvement in sensitivity.

21. CHEMO BOTANIC PROFILING OF CANNABIS PLANT EXTRACTS USING HPLC-UV, LC-MS AND GC-MS.

***Tijana Dautbegovic*, Mark Heatherington, Lawrence Hogge and Gordon Mckay. Pharmalytics Inc, University Of Saskatchewan, Saskatoon, SK.; Prairie Plant Systems, Saskatoon, SK**

Purpose: There is great interest in the medicinal properties of components found in plant material harvested from cannabis sativa. Critical to such studies is the characterization of the chemical constituents present. A common feature of this class of compounds is their thermal decomposition, which ultimately leads to production of the major stupefacient, delta 9-tetrahydrocannabinol (THC). The use of HPLC-UV, which avoids problems with thermal decomposition, was undertaken to aid in the chemo botanical identification of cannabinoids present in the plant materials from various cannabis spp. **Meth-**

ods: Dried plant material was ground in a food blender and extracted by shaking with methanol (MEOH). The MEOH was drawn off and the procedure repeated two more times. The MEOH extracts were combined and stored at 4°C for later analysis. An HPLC-UV method based on reverse phase chromatography with a C8 column and a mobile phase consisting of 65% acetonitrile: 35% water containing 0.04% formic acid was used. Extracts were analyzed by this system using a UV detector and a triple quadrapole mass spectrometer to further confirm peak identity. In addition, these same extracts were analyzed by GC-FID to quantify the THC content. **Results:** Depending on the species of cannabis plant material qualitative differences were observed for THC and its congeners including, THC acid, cannabinol, cannabindiol, cannabichromene and cannabigerol. The total THC content after GC-FID analysis ranged from less than 1% (hemp product) to more than 20% (cannabis sativa). The HPLC-UV procedure offers the opportunity to quantitate a variety of THC congeners without thermal decomposition whereas the GC-FID method only provides analytical data for THC. **Conclusions:** HPLC-UV can be used to chemically characterize the components present in various cannabis plant extracts. This information is critical to the final use of these products as potential therapeutic agents.

22. ACCURATE PREDICTION OF AQUEOUS SOLUBILITY.

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PURPOSE. For a potential drug to be effective orally, it must dissolve in an aqueous environment and be absorbed by the body. Aqueous drug solubility is the single largest factor driving passive human absorption via the gastrointestinal tract. Accurate prediction of aqueous solubility has been a long-sought-after goal among computational chemists: knowledge of a compound's solubility is essential for practical experimental reasons and to assess the possibility of passive gastrointestinal absorption. **METHODS**. In this paper, we describe the algorithm used at Advanced Chemistry Development (ACD) to produce a global method for the accurate prediction of aqueous solubility. Furthermore, we apply this software to a number of chemical datasets with experimentally available solubility data to validate this computational tool. **RESULTS**. Methodology and the software tool available from ACD allow evaluation of the aqueous solubility of a potential drug candidate at the early stages of the development process, prior to any analytical or synthetic work. Observed accuracy of prediction allows researchers to reveal the insoluble compounds within the libraries or synthetic sets and to eliminate them from further high-throughput screening processes. **CONCLUSION**. The newly developed software product makes ACD's highly accurate solubility prediction technology available to every medicinal and combinatorial chemist in a simple and intuitive desktop interface or through your company's informatics infrastructure.

23. CHROMATOGRAPHIC METHOD DEVELOPMENT: UTILIZING PRIOR CHROMATOGRAPHIC KNOWLEDGE AND PHYSICOCHEMICAL PREDICTION.

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PURPOSE. A common problem with chemistry-based organizations is the tendency for work to be repeated, often multiple times, due to an inability to impart complete information along with samples. Chromatographic method development is no exception to this rule. ACD/Method Development Suite combines chromatographic processing and prediction tools with a knowledge-based databasing capability. This combination expedites the chromatographic method development, which can now be based on chemical structures and retained chromatographic knowledge, and eliminates a substantial portion of repetitive experimental workload. **METHODS**. In this paper, we discuss the fundamentals of ACD chromatographic method development software and share experiences of its use for the creation of new HPLC methods. In addition to published chromatographic methods and vendor information, it creates archives of separations by organizations intended for internal use. The advent of accurate prediction of partition coefficients for ionizable species (Log*D*'s) presents an opportunity to predict retention times of compounds for various chromatographic systems using an initial characterization set. **RESULTS**. ACD/Method Development Suite is useful at a number of stages of method development, from creation of archives and evaluation of archived methods, to experiment-free prediction of elution order and retention times, and further to the "directed trial and error" of chromatographic Optimization Mode. The retention time prediction gives the chromatographer unprecedented ability to evaluate archived separation techniques prior to experimentation, and to modify conditions to optimize chromatographic conditions. **CONCLUSIONS**. The unique structural/experimental approach used in ACD/Method Development Suite gives the chromatographer an opportunity to use computation as an alternative to test injections and the associated waste of manpower, reagents, and instrument time.

24. ENZYMATIC DIGEST PEPTIDE ANALYSIS BY FULLY AUTOMATED HIGH PERFORMANCE 2D NANO-LC/ION TRAP MASS SPECTROMETRY.

Richard Smith **and Bernd Glatz; Agilent Technologies, 2660 Matheson Blvd. E., Mississauga, ON., Canada**

Purpose: For the structure elucidation of proteins in complex mixtures it has been shown that, 2D-HPLC in combination with ion trap mass spectrometry significantly increases protein identification capacity. This presentation will describe a 2D nano-HPLC/ion trap mass spectrometer system and how this combination can be used to generate peptide MS and MS/MS data allowing the identification of proteins in complex mixtures to be accomplished. **Method**: Nano-HPLC in combination with nanoelectrospray MS/MS with an ion trap mass spectrometer allows reliable detection of fmole to amole amounts of peptides from enzymatic protein digests. The bottlenecks of these techniques are high operational skills and the general robustness of the system. For example, reliable gradient delivery of flow rates in the 100nL/min range, combined with small delay volumes for true gradient profiles, low dispersion connections and the overall automation capability of the complete system are essential for a high performance yet robust system. **Results**: MS with data-dependent automatic scan functions including Auto-MS^5, ActiveExclusion and MaxResScan, as well as a fully automated protein database search (MASCOT) complete the identification of enzymatic peptide fragments and the overall protein identification with high sequence coverage. **Conclusion**: Altogether, we describe a concept/system, which combines the robustness of classical HPLC with the sensitivity improvements of nano-HPLC and nanoelectrospray ion trap mass spectrometry.

25. CYCLOSPORIN (CSA) RELEASE FROM MICROEMULSION: EFFECT OF MEMBRANE APPOSITION.

Dody Reimer**, Rick Smith, Llloyd Jeffs, Kim Wong, Thomas Redelmeier; Northern Lipids Inc.; Department Of Biochemistry & Molecular Biology, University Of British Columbia, Vancouver, B.C., Canada**

Purpose: To develop an assay to evaluate the release of cyclosporin A (CsA) from emulsions and to characerize the influence of lipid composition on the release of this drug. **Methods:** A novel lipid-based carrier system containing a triglyceride core with CsA was developed and characterized by FFEM and ^{31}P-NMR. The *in vitro* drug release from donor emulsions was evaluated following exchange into EPC/EPG "acceptor" liposomes. Donor emulsions were prepared by combining CsA (1/50, wt/wt) with tricaprylin, surfactants and co-solvents. ^{3}H CsA was added to the oil phase to achieve no greater than 3 μCi/ml in the final formulation. The mixture was diluted with 30% Ethanol and dialyzed against 300 mM sucrose to remove solvents. Liposome acceptor vesicles [EPC/EPG (9/1) or EPC/EPG/Peg$_{2000}$DSPE (85/5/10, mol/mol/mol)] were prepared in 300 mM sucrose at 50 mg/ml by extrusion through 100 nm polycarbonate filters. Donor emulsions and acceptor liposomes were incubated at ambient temperature at fixed donor/acceptor ratios and DEAE-Sephacel ion exchange chromatography was used to separate the donor vesicles from the acceptor vesicles. Aliquots of eluted fractions were counted for ^{3}H CsA and the amount of drug released from the emulsion fraction was determined. The effect of donor/acceptor vesicles and the surfactant composition were evaluated for their effect on the kinetics of CsA release. **Results and Conclusion**: FFEM shows that the particles with triglyceride cores have a smooth cross-fracture face and is typical of that seen for emulsions. ^{31}P-NMR studies on this emulsion demonstrate an isotropic signal that is typical of phospholipids in small (< 200 nm) structures. The addition of a signal-broadening agent (MnSO$_4$) quenched the signal leading to our conclusion that the phospholipid was present in a monolayer. These observations suggest that hydrophobic drugs are released from membranes by collision with "acceptor" membranes and not by diffusion through the aqueous phase. CsA was not released from emulsions upon dialysis against a buffer containing acceptor vesicles. In contrast, CsA was rapidly released from emulsions when incubated with "acceptor" vesicles consisting of EPC:EPG. Drug release was dependent on the donor/acceptor ratio and the lipid composition of the acceptor vesicle. All CsA was available for exchange into the liposomes. Altering the surfactant composition of the emulsion formulations did not significantly influence the rate of CsA release. However, the inclusion of Peg$_{2000}$DSPE in the acceptor vesicles significantly reduced both the rate and extent of CsA exchange, confirming that membrane apposition facilitated drug release from the donor emulsion.

25a. ALBUMIN FREE PREPARATIONS OF ERYTHROPOETIN

Janette Cruz-Rodríguez**, Dayamí Villalta Blen, Center of Molecular Immunology, Cuba.**

Erythropoietin (EPO) is an acidic glycoprotein hormone that promotes the differentiation and proliferation of erythroid progenitor cells. EPO plays a central role in maintaining such homeostasis of erythrocytes in the living organism. EPO manufactured by recombinant DNA technology has the same biological effects as endogenous hormone, and its use in biotechnology industry has been successful. Drug design for supplying the market with stable recombinant human EPO preparations requires that chemical changes (hydrolysis, disulphide exchange reactions, etc) or physical change (denaturation, aggregation, adsorption, etc) to be suppressed. Nowadays, most of marked r-HuEPO contains Human Serum Albumin (HSA). The worldwide questioned addition of a blood-derived protein, like HSA, to a drug formulation force to find a new stabiliser, such as our founded amino acids mixtures. r-HuEPO solution preparations, containing various amino acids in sodium phosphate buffered solution, were prepared and subjected to an accelerated testing conducted for days at 60°C and a preliminary test at real temperature during 6 months. SDS-PAGE, reverse-phase HPLC, gel-filtration HPLC and biological activity measured the r-HuEPO content in each of the preparations after the test. Quantitative analyses of the HPLC results were made. Peak area log vs. time of the data corresponding to each sampling time for each preparation was graphed. Then a lineal regression analyse was make to get the constant degradation value (k). The results demonstrated selected amino acids to be effective in suppressing the formation of EPO degradation products to be observed in the preparations after the stability testing.

26. DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF CELECOXIB AND ROFECOXIB ANALOGS AS SELECTIVE COX-2 INHIBITORS: REPLACEMENT OF SULFONAMIDE AND SULFONYLMETHYL PHARMACOPHORES BY A SULFONYLAZIDE BIOISOSTERE

M.J. Uddin, **P. N. Praveen Rao, E. E. Knaus; Faculty of Pharmacy, University Of Alberta, Edmonton, Canada**

Purpose: The discovery that cyclooxygenase-2 (COX-2) is an inducible isozyme, which causes inflammation, and that the constitutive isozyme COX-1 provides gastroprotection, provided the rationale for design of the non-ulcerogenic selective COX-2 inhibitors celecoxib and rofecoxib. In an earlier study, we showed that the dipolar azido pharmacophore (**1b**, **2b**) was a suitable bioisostere of the sulfonamide moiety in celecoxib (**1a**), or the methylsulfonyl moiety in rofecoxib (**2a**) [*J. Med. Chem.*, **44**, 3039-3042, (2001)] that inserts deep into the 2°-pocket of COX-2. The azido group undergoes strong electrostatic interaction with Arg^{513}, whereas the sulfonamide and methylsulfonyl groups are only capable of H-bonding interactions. This study has now been extended to include the hitherto unknown SO_2N_3 dual binding pharmacophore (both H-bonding and ion-ion interactions). **Methods: a) Chemistry:** The SO_2N_3 analogs of celecoxib (**1c-d**), and rofecoxib (**2c-d**) were synthesized by modification of procedures originally developed for the preparation of the azido analogs (**1b**, **2b**). **b) In vitro enzyme inhibition:** Compounds were evaluated for their ability to inhibit (IC_{50}, μM values) COX-1 and COX-2, and the COX-2 selectivity index were calculated. **c) Molecular Modeling (Docking) Studies:** The lowest energy conformation of the test compound obtained after dynamics optimization was docked in the COX-2 binding site to investigate the binding interaction.

Results: Preliminary docking studies showed that compound **1d** binds effectively in the COX-2 binding site with an intermolecular binding energy higher than that observed for celecoxib. The fact that the SO_2N_3 of **1d** is inserted deep inside the entrance to the 2°-pocket of COX-2 suggests the hypothesis presented is valid, and that the SO_2N_3 group, will be a suitable replacement (bioisostere) for the H-bonding SO_2NH_2 and SO_2Me pharmacophores. Compound **1d** selectively inhibited COX-2 (COX-1 IC_{50} = 5.15 μM; COX-2 IC_{50} = > 100 μM) for a COX-2 selectivity index > 19.4. **Conclusion**: It has been demonstrated that the sulfonylazido compound **1d**, in which the respective sulfonamide pharmacophore present in celecoxib is replaced by a sulfonylazide bioisostere, is a selective COX-2 inhibitor. Molecular modeling (docking) studies showed that the sulfonylazido group of **1d** inserts deep into the secondary pocket of COX-2 where it undergoes both H-bonding and electrostatic ion-ion interactions with Arg^{513}. These results indicate that the sulfonylazide is a potentially novel COX-2 dipolar dual H-bonding/electrostatic pharmacophore for the design of selective, and potent, COX-2 inhibitors.

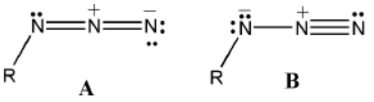
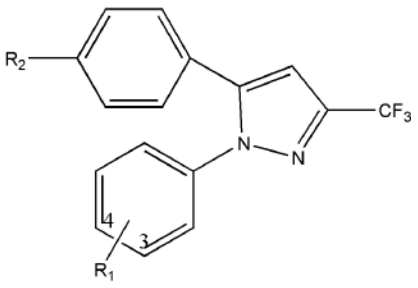
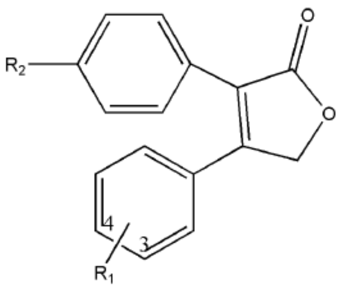


Figure 1. Two major azide resonant species.



1a, R_1=4-SO_2NH_2, R_2=Me (Celecoxib)
1b, R_1=4-N_3, R_2=Me
1c, R_1=4-SO_2N_3, R_2=Me
1d, R_1=3-SO_2N_3, R_2=Me



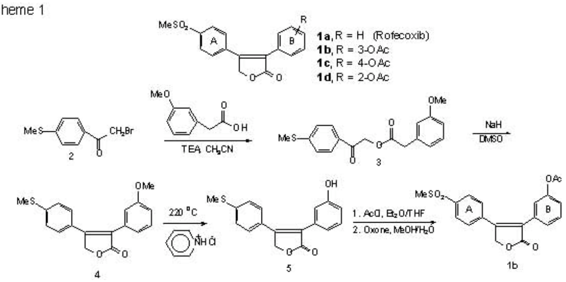
2a, R_1=4-SO_2Me, R_2=H (Rofecoxib)
2b, R_1=4-N_3, R_2=H
2c, R_1=4-SO_2N_3, R_2=H
2d, R_1=3-SO_2N_3, R_2=H

27. DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF ROFECOXIB ANALOGS AS ACETYLATING SELECTIVE COX-2 INHIBITORS.

M.A. Rahim, P. N. Praveen Rao and *E.E. Knaus*; Faculty Of Pharmacy, University Of Alberta, Edmonton, Alberta

Purpose: Aspirin (ASA), a traditional NSAID acetylates both COX-1 and COX-2, being 10-to-100-fold more potent against COX-1. Acetylation of the weakly nucleophilic OH of Ser^{530} in the COX binding site by ASA is thought to result from initial binding of its *ortho*-CO_2H to an arginine residue at the mouth of the COX channel that positions the acetoxy moiety near the Ser^{530} OH, which it acetylates. In view of the beneficial effects of ASA in preventing thrombosis and platelet aggregation, it is proposed that a highly selective COX-2 inhibitor such as rofecoxib possessing an appropriately positioned complimentary aryl acetoxy moiety (B-ring) would preferentially acetylate the COX-2 Ser^{530} acetylation site. The phenyl ring **A** having the $MeSO_2$ pharmacophore is suitably positioned such that its $MeSO_2$ group can insert deep inside the entrance of the associated secondary COX-2 binding pocket. The primary objective of this project involves the design of a 2nd-generation class of COX-2 inhibitor that can also acetylate the enzyme (like ASA) to determine if this is a useful drug design concept that may be therapeutically beneficial. **Methods**: **a) Chemistry:** The acetoxy derivatives (**1b-d**) of rofecoxib (**1a**) were prepared using a final 3-step process (R = OMe → OH → OAc) as shown in Scheme 1. **b) *In vitro* enzyme inhibition:** Compounds were evaluated for their ability to inhibit (IC_{50}, μM values) COX-1 and COX-2 using a COX-ovine inhibitor screening kit (Cayman Chemicals), and the COX-2 selectivity index was calculated; **c) Anti-inflammatory Assay:** Test compounds were evaluated using the *in vivo* rat carrageenan-induced paw edema assay; **d) Analgesic Assay:** Analgesic activity was determined using the 4% sodium chloride-induced writhing (abdominal constriction) assay.

Scheme 1

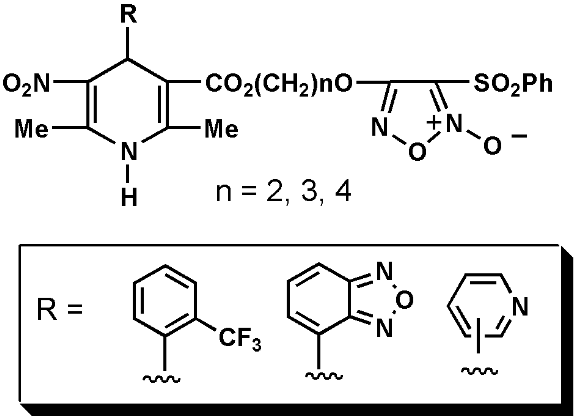


Results: Efficient synthetic methods were developed for the preparation of compounds **1b-1d** (Scheme 1) that are highly selective COX-2 inhibitors (COX-2 selectivity indices > 28,482). *In vivo* anti-inflammatory (AI) and analgesic assays were performed, the *meta*-OAc substituted compound **1b** is highly potent (AI activity at 3 hrs = 47.9 ± 0.7% for a 20 mg/kg oral dose; analgesic activity at 1 hr = 52.4 ± 4.9% for a 50 mg/kg ip dose) .**Conclusions**: We have developed a new synthetic method for the preparation of novel acetyl substituted 3,4-diaryl-2(5H)-furanone derivatives(**1b-1d**) that are highly selective COX-2 inhibitors. *In vivo* AI and analgesic data showed that the meta-OAc compound **1b** exhibits both analgesic and AI activities.

28. NITRIC OXIDE DONOR FUROXANYL 1, 4-DIHYDROPYRIDINES AS CALCIUM CHANNEL MODULATORS TO TREAT CONGESTIVE HEART FAILURE.

J-T. Nguyen, D. Vo, R. Shan, and *E.E. Knaus*; Faculty Of Pharmacy and Pharmaceutical Sciences, University Of Alberta, Edmonton, Alberta, Canada.

Purpose: The development of tissue selective 1,4-dihydropyridine L-type calcium channel modulators for the treatment of congestive heart failure dictates that their adverse smooth muscle vasoconstrictor action be abolished and/or separated from the desired target cardiac calcium channel agonist (positive inotropic) stimulant effect. Organic nitrovasodilators that exert their effect in vivo by bypassing the nitric oxide-production system in the endothelium to deliver nitric oxide (•NO) directly to muscle cells in the artery has prompted us to design hybrid Hantzsch-type 1,4-DHP calcium channel modulators that have the ability to simultaneously release •NO. **Methods:** A group of racemic 1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridinecarboxylates possessing either a C-4 2-trifluoromethylphenyl, benzofurazan-4-yl, 2-pyridyl, 3-pyridyl, or 4-pyridyl, substituent in conjunction with a •NO donor C-3 ester [3-(benzenesulfonyl)furoxan-4-yloxy)alkyl substituent were synthesized using modified Hantzsch reactions. Smooth muscle calcium channel antagonist activity on guinea pig ileum longitudinal smooth muscle (GPILSM) was determined. Cardiac calcium channel agonist activity was calculated as the percentage increase (positive inotropic effect) in contractile force of isolated guinea pig left atrium (GPLA) relative to its basal contractile force in the absence of the test compound. In vitro •NO release was measured using the Griess reaction in the absence and presence of L-cysteine (3.2 mM). **Results:** Compounds having a C-4 2-, 3- or 4-pyridyl substituent exhibited more potent in vitro calcium channel antagonist activity (IC_{50}'s in the 10^{-6} to 10^{-7} M range) on GPILSM than related analogs having a C-4 2-trifluoromethylphenyl or benzofurazan-4-yl subsitutent (IC_{50} = 2.99 × 10^{-5} M). The point of attachment of the pyridyl ring (2-, 3- or 4-), and the length of the C-3 ester alkyl spacer [$-CH_2(CH_2)_n$, n = 1-3), were not determinants of smooth muscle calcium channel antagonist activity. Compounds having a C-4 3-pyridyl, or 4-pyridyl, substituents were the most potent cardiac positive inotropes (EC_{50}'s in the 10^{-6} to 10^{-7} M range). The % nitric oxide released in vitro in the presence of L-cysteine for this group of compounds was higher (36-74% range) than that for the reference drug glycerol trinitrate (20%). A quantitative structure-activity analysis showed an inverse correlation between a molecular weight descriptor and % nitric oxide released. **Conclusion:** Model hybrid (calcium channel modulation, nitric oxide donor) compounds, that show dual cardioselective agonist (positive inotropic) / smooth muscle selective antagonist activities, constitute a novel type of 1,4-DHP calcium channel modulator which provides a potential drug design concept targeted toward the treatment of congestive heart failure, and as useful probes to study the structure-function relationship of calcium channels.



29. INFLUENCE OF A NOVEL HYDROPHILIC PHYTOSTANOL COMPOUND ON THE TRANSPORT AND ACCUMULATION OF CHOLESTEROL CONTAINING MICELLES WITHIN THE HUMAN EPITHELIAL CELL LINE CACO-2.

Manisha Ramaswamy, Ming, Li, Kathy Boulanger, P. Haydn Pritchard and *Kishor Wasan*; University Of British Columbia, Vancouver, B.C., Canada

Purpose. The objective of this study was to determine the effects of a novel hydrophilic phytostanol (FM-VP4) on the transport and accumulation of cholesterol within Caco-2 cell monolayers. **Methods.** The transepithelial transport from apical to basolateral side and cellular accumulation of cholesterol containing micelles in the presence and absence of FM-VP4 were measured using Caco-2 cell monolayers grown in Transwell chambers. Stock solutions of cholesterol containing micelles were prepared by dissolving cholesterol (hot + cold) in a chloroform/methanol (10:1 v/v) mixture. [^{3}H]cholesterol, sodium taurocholate (NaTC), and oleic acid were dissolved in ethanol. Appropriate aliquots were taken from each stock solution, dried under nitrogen and resuspended in a buffer solution to give a final concentration of 50 µM cholesterol (hot + cold), 5mM NaTC and 400µM oleic acid. Micelle formation was confirmed by electron microscopy. [^{3}H]Cholesterol containing micelles (50 µM) were co-incubated with 50 µM FM-VP4 dissolved in tissue culture media at 37°C in 5% CO2 for 4 h and the percent [^{3}H]cholesterol cellular accumulation and transport were determined by radioactivity. **Results.** [^{3}H]Cholesterol accumulation within Caco-2 cell monolayers in the presence of FM-VP4 (50 mM) was significantly less compared to controls following 4 h of incubation (Controls, 59.8 ± 5.2% vs. FM-VP4, 33.7 ± 7.0% of original [^{3}H]cholesterol incubated, n=3; $p<0.05$). [^{3}H]Cholesterol transport from the apical to basolateral side was less than 1% of the original concentration incubated in FM-VP4 and control treatment groups. Total [^{3}H]cholesterol recovery for all studies was greater than 92%. **Conclusion.** FM-VP4 co-administration inhibited [^{3}H]cholesterol accumulation within Caco-2 cell monolayers without influencing its transport from the apical to basolateral side. **Acknowledgements:** *CIHR-Forbes Medi-Tech Inc. University-Industry Grant* Previously Presented at the 2001 AAPS Meeting, Denver CO.

30. PHYTOSTANOL PHOSPHORYL ASCORBATE IS AN EFFECTIVE NOVEL HYPOCHOLESTEROLEMIC AND ANTIATHEROSCLEROTIC AGENT.

***P.Haydn Pritchard*, Tatjana Lukic, Kish M Wasan; Forbes Medi-Tech Inc.and University Of British Columbia Vancouver, BC Canada**

Purpose: To evaluate the effectiveness of FM-VP4; an inhibitor of cholesterol absorption. **Methods:** FM-VP4 was added to the diet or drinking water of experimental animals for the indicated times. Blood lipids were measured at intervals. **Results:** Gerbils – Consumption of 2% FM-VP4 resulted in a 55.7% and 50.7 % reduction in TC after 4 and 8 weeks. This reduction was entirely due to the loss of the LDL pool, which was reduced to undetectable levels at both time points. At 8 weeks, the HDL levels had risen by 33.8 % whereas TG levels had decreased by 60.2%. Apo E-deficient mice – FM-VP4 caused a 73% and 74% decrease in TC compared to controls after 4 and 8 weeks. This was associated with a 50% and 82% decrease in TG. After 12 weeks, assessment of atherosclerotic lesions in aortic roots demonstrated a 75% decrease in lesion area involvement. Dogs - FM-VP4 significantly lowered TC in normocholesterolemic dogs (54% in males, 44% in females). At a dose of 250mg/kg/d, TC was reduced by approximately 20% in both sexes. In a second study in normocholesterolemic dogs using an improved formulation of FM-VP4, we observed that a dose of 250mg/kg resulted in a 43% reduction in the first 5 days of dosing. **Conclusions:** Taken together, these results show compelling evidence that FM-VP4 is a candidate new drug with significant hypolipidemic potential.

31. THE INFLUENCE OF LIPID TRANSFER PROTEIN I (LTP I) FACILITATED PHOSPHATIDYLCHOLINE (PC) TRANSFER ACTIVITY ON CYSLOSPORINE A (CSA) TRANSFER BETWEEN LOW-DENSITY AND HIGH-DENSITY LIPOPROTEINS.

Mona Kwong, **Kishor M. Wasan**

Purpose. The purpose of this study was to determine if lipid transfer protein I (LTP I) facilitated phospholipid (PC) transfer activity regulates the plasma lipoprotein distribution of cyclosporine A (CSA). **Methods**. To assess if LTP I facilitated PC transfer activity regulates the plasma lipoprotein distribution of CSA the following experiments were completed. ^{14}C-PC or ^{3}H-CSA-enriched high-density lipoproteins (HDL) or low-density lipoproteins (LDL) were incubated in T150 buffer [50mM Tris-HCl, 150mM NaCl, 0.02% sodium azide, 0.01% disodium ETDA, pH 7.4 which contained a ^{14}C-PC or ^{3}H-CSA-free lipoprotein counterpart +/- exogenous LTP I (1.2 mg protein/ml)] or in delipidated human plasma which contained 1.2 mg protein/ml of endogenous LTP I in the presence or absence of a monoclonal antibody TP1 (30 mg protein/ml) directed against LTP I for 90 minutes at 37^0 C. The fraction of PC and CSA transferred (%kt) were calculated as follows: %kt = -ln $(1-A_t/D_o)*100$ where D_o and A_t are radioactivity of donor and acceptor at time zero respectively. Constant k is fraction of labelled transferred per unit time, t. **Results**. The percent transfer of PC from LDL to HDL was different from the percent transfer of CSA in T150 buffer [PC transfer, 6.33 ± 0.3 %kt (n=4) vs. CSA transfer, non-detectable (n=4)] or human plasma source [PC transfer, 24.26 ±2.99 %kt (n=5) vs. CSA transfer, non-detectable (n=5)]. In the presence of TP1, only PC transfer from LDL to HDL decreased. Non-detectable CSA transfer was observed. **Conclusions**. These findings suggest that the transfer of CSA between different lipoprotein particles is not influenced by LTP I facilitated PC transfer activity. **Acknowledgements**. Funding from Canadian Institutes of Health Research (Grant #MT-14484) Presented in American Association of Pharmaceutical Scientists Annual Meeting 2001, Denver, CO. Published Abstract AAPSPharmSci, Volume 3, No. 3, 2001

32. AEROSOL DELIVERY OF LIPOSOMAL CIPROFLOXACIN IN DRY POWDER FORM.

Tejas Desai, **Jonathan P. Wong, and Warren H. Finlay; Aerosol Research Laboratory Of Alberta, Department Of Mechanical Engineering, University Of Alberta, Edmonton, Alberta, Canada; Defense Research Establishment Suffield, Box 4000, Medicine Hat, Alberta**

Purpose: Dry powder liposome formulations are conventionally prepared by lyophilization of the aqueous liposome dispersions, followed by micronization to the size of 1-5µm by jet-milling. In our previous study, we demonstrated the detrimental effects caused by lyophilization and jet-milling to the liposome integrity (1). In an attempt to circumvent these deleterious effects, the feasibility of formulating phospholipid-based powders that result in spontaneous formation of liposomes in the aqueous environment has been investigated in this study. The potent antimicrobial agent ciprofloxacin was selected as a model drug to demonstrate this approach. **Experimental:** The concept is demonstrated by formulating various compositions containing phospholipids(s), drug and lactose, and dispersing them in saline. Ciprofloxacin was added at a dosage of 7mg/ml. The entrapment efficiencies of different compositions were determined by mixing appropriately weighed formulations (to achieve the desired concentrations), and dispersing in saline by gentle mixing at a room temperature (RT) of 24±1°C. The phospholipids were then allowed to hydrate by equilibrating the dispersions for 15 minutes at RT. The entrapment efficiencies were determined as described earlier (1). Phospholipids such as egg phosphatidylcholine (EPC), Dipalmitoyl phosphatidylcholine (DPPC), dimyristoyl phosphatidylglycerol (DMPG) (sodium salt) were used in this study. Formulations with combinations of (EPC+Cholesterol) and (EPC+DMPG) were prepared at a molar ratio of 1:1. Lactose was added at a molar ratio of 1:5 (lipid/ lactose). **Results:** The effect of various phospholipids on the entrapment of ciprofloxacin is shown in Figure 1.

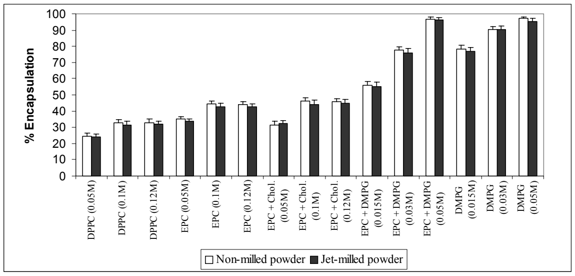


Figure 1: *Entrapment efficiency (%) of ciprofloxacin in liposomes formed upon dispersion of various formulations consisting of different lipids at*

various concentrations. (n=3, Error bars indicate SD) (Reproduced with permission from Desai et al. J. Pharm. Sci., 91, 482-491, 2002)

The results indicate the spontaneous formation of liposomes upon hydration and subsequent partitioning of ciprofloxacin from the aqueous phase into the vesicular structure. Micronization of these powders by jet-milling did not cause appreciable leakage of ciprofloxacin. Figure 2 shows electron microscopy of freeze-fractured surfaces derived from EPC + DMPG (1:1) based formulations encapsulating ciprofloxacin.

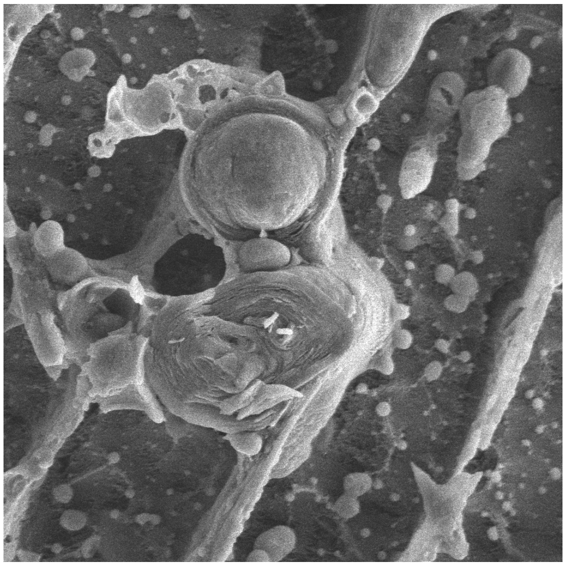


Figure 2: *Freeze fractured electron micrograph of liposomes derived from (EPC + DMPG) mixture (Reproduced with permission from Desai et al. J. Pharm. Sci., 91, 482-491, 2002)*

The micrograph indicates that multi-lamellar vesicles (MLVs) are formed upon hydration of phospholipids, which act as reseroir for encapsulating drugs. **Conclusions:** We have developed a novel method to overcome the deleterious effects of lyophilization and jet-milling when delivering liposomes in dry powder aerosol form. Application of this method shows dramatic reductions in the drug leakage, from 60%-80% leakage with a conventional approach to no measurable leakage with our approach. Preliminary experimental data of aerosol dispersion also indicate that these formulations have excellent powder dispersibility (unpublished data), so that this approach is attractive for inhaled aerosol delivery of liposomes. **References:** Desai, TR, Wong, JP, Hancock, REW, Finlay, WH., (2002) A Novel Approach to the Pulmonary Delivery of Liposomes in Dry Powder Form to Eliminate the Deleterious Effects of Milling. J. Pharm. Sci., 91, 482-491.

33. IN VITRO GENERATION AND ANALYSIS OF NEBULIZED NIOSOMAL ALL-TRANS-RETINOIC ACID.

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Purpose: All trans-retinoic acid (ATRA) and its derivatives are potentially useful therapeutic agents for lung cancer and malignant disorders (1). However, chronic administration of retinoids in patients is associated with toxic effects (2). Incorporation of such therapeutic agents in colloidal carriers, such as liposomes, is known to reduce its toxic effects and change their pharmacokinetics. However, the high cost and variable purity of natural phospholipids have raised concerns over the adoption of liposomal drug delivery systems. An alternative approach that may overcome these problems involves the formation of liposome-like vesicles from nonionic surfactants commonly referred to as niosomes. However, the feasibility of aerosolizing niosomes has not been explored yet. In this study, we demonstrate the possibility of delivery of niosomal ATRA by nebulization. **Methods**: **Preparation of niosomes**: Niosomes were prepared by dissolving the surfactants and ATRA in chloroform. The solvent was evaporated under vacuum. The film was then hydrated with 0.9% saline, and the dispersion was sonicated in an ultrasound bath. The dispersion was then equilibrated at room temperature for 1 hour. Free drug was removed by centrifugation to achieve an encapsulation efficiency of >95%. The encapsulation efficiency was determined by measuring the amount of ATRA in the supernatant and residue by UV absorption at 350nm using a UV Spectrophotometer. **Nebulization of niosomal ATRA**: Niosomal ATRA was nebulized with Pari LC STAR jet nebulizers, using a DeVilbiss Pulmo Aide Compressor. The aerosol was collected on Respigard filters. The filter contents were extracted with 0.9% saline. A portion of the sample was then centrifuged to determine the encapsulation efficiency as described earlier. **Particle size measurement of the nebulized niosomes**: Particle size distribution was determined by connecting nebulizers to an Andersen cascade impactor and running the nebulizers intermittently to avoid droplet shrinkage due to cooling in the impactor. The impactor flow rate was calibrated to 28.3 l/min. The MMADs of aerosol droplets were determined by extracting the content of each plate and assaying for ATRA. To determine the entrapment efficiency of ATRA on the various stages of the impactor, the fractions collected on each plate were extracted with 0.9% saline. These extracts were then centrifuged and the resulting supernatant and pellet were assayed separately for ATRA. **Results**: Among various formulations prepared, the formulation shown in Table 1 exhibited excellent behavior upon nebulization.

Table 1: Formulation used for nebulization of niosomal ATRA

Component	Concentration (mg/ml)
Span 20	13.24
Tween 80	8.12
ATRA	1.00

Values of various parameters for this formulation are shown in Table 2.

Table 2: Comparison of various parameters following nebulization of niosomal formulation (Mean ± SD, n=3):

Parameter	
Nebulization efficiency (%)	36.9+2.6
Entrapment of ATRA in nebulized niosomes (%)	73.4+1.9
Entrapment of ATRA in unnebulized dispersion (%)	89.0+0.9
Mass median aerodynami diameter (MMAD), µm	3.7+0.3
Geometric standard deviation (GSD)	1.59+0.17

Figure 1 shows the distribution of total and encapsulated drug on each stage of the impactor for this formulation.

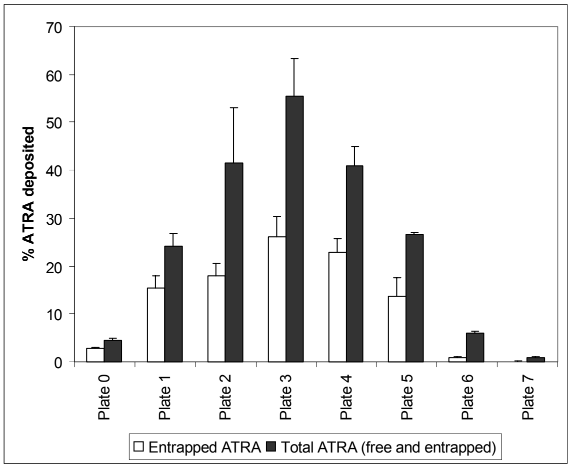

Figure 1: *Deposition of niosome entrapped and total ATRA in the Anderson cascade impactor following nebulization of niosomal formulation.*

Independent assessment of the size distribution of the aqueous phase and the niosomes in aerosols showed no statistically significant difference (P=0.87) in their MMADs, indicating that the drug is homogeneously distributed in the aerosol droplets. **Conclusions**: We have demonstrated that optimized niosomal formulations can be nebulized efficiently with good entrapment of ATRA (>50%) in aerosol droplets of appropriate size for inhalation therapy. This opens the door for future in vivo testing of the respiratory delivery of niosomal-ATRA formulations for lung cancer treatment. **References**: Lokshin, A., Zhang, H., Mayotte, J., Lokshin, M., Levitt, M.L., Anticancer Research, 19, 5251-5254, 1999. Lippman, S.M., Kessler, J.F., Meyskens, F.L., Cancer Treatment Reports, 71, 493-515, 1987.

34. BLOCK COPOLYMER MICELLES FOR THE ENCAPSULATION AND DELIVERY OF AMPHOTERICIN B*.

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Purpose: To assess the effect of fatty acid substitution of a micelle-forming poly (ethylene oxide)-*block*-poly (*N*-hexyl stearate-L-aspartamide) (PEO-*b*-PHSA) on the encapsulation, haemolytic properties and antifungal activity of amphotericin B (AmB). **Methods:** PEO-*b*-PHSA with three levels of stearic acid substitution were synthesized and used to encapsulate AmB by a solvent evaporation method. Size exclusion chromatography and UV spectroscopy were used to confirm and measure levels of encapsulated AmB. The haemolytic activity of encapsulated AmB toward human red blood cells and its minimum inhibitory concentration against *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans* were obtained and compared to AmB alone. **Results:** An increase in the level of stearic acid substitution on PEO-*b*-PHSA improved the encapsulation of AmB while reducing its haemolytic activity. PEO-*b*-PHSA micelles having 50 and 70 % stearic acid substitution (mol fatty acid: mol reacted and unreacted hydroxyls) were completely non-haemolytic at 22 µg/ml. At 11 % stearic acid substitution, AmB caused 50 % haemolysis at 1 µg/ml. AmB in PEO-*b*-PHSA micelles was as effective as AmB alone against pathogenic fungi. **Conclusions:** PEO-*b*-PHSA micelles with a high level of stearic acid side chain substitution can effectively solubilize AmB, reduce its haemolytic activity yet retain its potent antifungal effects. *This poster has already been presented in the annual meeting of American Association of Pharmaceutical Scientists (2001) in Denver, Colorado. The full manuscript is in press in *Pharmaceutical Research*.

35. IMPROVED CANCER THERAPY THROUGH FOLATE CONJUGATED MITOMYCIN 'C' LOADED MEMBRANE VESICLES

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Purpose In the past few decades, a number of advances took place in the methodologies to prepare an erythrocyte-based delivery system. In our earlier report, we prepared a new erythrocyte-based carrier system loaded with doxorubicin HCl that that is small, natural, nontoxic, nonimmunogenic, and readily available in large quantities. These vesicles were found quite stable and results indicated that liver and spleen remained the major organs for clearance of these vesicles while at the same time the toxicity in the heart was almost reduced (1). To solve the problem of site specific targeting an attempt has been made by coating erythrocytes with small ligand (folic acid) for tumor targeting. Folic acid is a well known vitamin, which are over expressed on the surface of cancer cells (2). Therefore, this receptor has been identified as a tumor marker. Thus, a new concept has been approached by coating the membrane vesicle with folic acid for improved cancer therapy. **Methods Materials** Mitomycin C (MMC) was received as a gift sample from M/s Biochem Industries, Mumbai, India; NHS-folate(Sigma, USA); Sodium Phosphate; Potassium dihydrogen orthophosphate, Sodium chloride; (Ranbaxy Lab Delhi, India); Dimethyl formamide (DMF); and Heparin sodium (Biological Evans, Mumbai, India). **Preparation of folate conjugated MMC loaded membrane vesicles** The membrane vesicles from erythrocyte ghosts were prepared by induced endocytosis (3,1) by mixing with 35 ml of cold vesiculating medium ontaining Tris EDTA (pH 8.5) and Tris HCl (optimized parameters) in centrifuge tubes, kept for 30 min at 4°C and then for 15 min at 37°C and allowed to undergo spontaneous vesiculation. The pellets were resuspended in sodium phosphate buffer (pH 8.0) containing final drug (MMC) solution (5 mg/ml) and then vesiculation was completed by five passes through 27-gauge needle. These drug-loaded vesicles (MMC-V) were purified using dextran barrier. Modification with folic acid (MMC-FV) was carried out by adding one ml of succinylated BSA (equivalent to NHS-folate concentration) to the vesicle suspension and shaken for five minutes at room temperature. Finally, different concentrations of NHS-folate were added to the mixture followed by incubation at 37°C for one hour. **In-vitro Characterization** The prepared membrane vesicles were characterized for substitution level of folate molecules, entrapment efficiency, in-vitro drug release and stability studies employing appropriate methods. In-vitro Cytotoxicity and cytostatic assay were carried out using Murine leukemia L-1210 cells. Cellular sensitivity to the drug was defined as the mean IC_{50}, which is the concentration of MMC required to inhibit growth of 50 % of the untreated cell population. The antitumor activity of the surface modified membrane vesicles against L-1210 cells was assessed using Balb/c mice (25-30 gm.). Mice were daily treated from day 1 to day 6 i.p. injection of free drug, FV (Plain folate treated vesicles), MMC-V (mitomycin 'C' loaded vesicles) and MMC-FV (folate conjugated mitomycin 'c' loaded vesicles) at a dose of 2 mg/kg body weight. Median survival times (MST) for each group containing 6 mice was noted with reference to control. **Results** The method of preparation of membrane vesicles using induced endocytosis is based on budding of membrane into the ghost's interior leading to accumulation of small vesicles within each parent ghosts. During vesicle formation, the drug solution present is entrapped in the vesicles. The amount of MMC entrapped was about 0.58 mg/mg of vesicle protein. To generate vesicles, erythrocyte ghosts are lysed and incubated in the vesiculating medium. On treatment with 100, 200, and 300 µg of NHS-folate, approximately 73, 112, 129 ng were bound respectively per µg of vesicle protein. In-vitro release of drug from MMC-V and MMC-FV were 43.4% and 37.1% respectively after 16h, which may be attributed to the barrier posed by folate molecules which is covalently coupled to amino group of the vesicles. The size of the vesicles thus obtained was in the range of 1.2-1.5µm. No significant leakage and aggregation was observed at selected temperature over storage time of 21 days, which reveals that formulations are quite stable. The order of cytotoxicity was MMC-FV > MMC-V > free drug. The number of viable cells was reduced with time. The MST (median survival time) was found in order of MMC-FV>MMC-V>free drug>control (FV) (Table 1).

Table 1 Antitumor activity of various vesicle formulations in Balb/c mice bearing murine leukemia L-1210.

Formulation	MST±s.d. (days)	ILS (T/C %)	Survival
PBS soln	17.8±3.2	100.00	0/6
FV	17.7±2.4	99.43	0/6
Free drug	23.6±1.7	132.58	1/6
MMC-V	29.8±1.8	167.41	3/6
MMC-FV	39.4±2.3	221.14	5/6

ILS = Increase in life span; MST = Median survival Time; s.d.= Standard deviation. FV=Folate conjugated plain vesicles, MMC-V= Mitomycin 'C' loaded membrane vesicles, MMC-FV=Folate conjugated Mitomycin 'C' loaded membrane vesicles.

The ILS for MMC-FV was 221.14%. From these data, it can be concluded that the MST in mice was found to depend mainly on two factors; sustained release effect, and direct interaction of tumor cells with formulation.(3). **Conclusion** This new approach of surface modification of erythrocyte ghost derived membrane vesicles using folic acid is relatively simple and can be used to direct towards cancer cells. Thus, the prepared MMC loaded membrane vesicles conjugated with folic acid represent a new potential delivery system for improved cancer therapy. **References** 1. P.R Mishra. and N.K Jain Reverse biomembrane vesicles for effective controlled delivery of Doxorubicin Drug Delivery, **7** 153-159 (2000). 2. S.D Weitman, R.H Lark, L.R Coney, D.W Fort, V Frasca, V.R.J, Zurawskiand B.A.Kamen Distribution of the folate receptor GP 38 in normal and malignant tissues. Cancer Res, **52(12)** 3396-3401 (1992). 3. J.J. Turek, C.P. Leamaon, and P.S.Low Endocytosis of folate protein conjugate, ultrastructural localization in KB cells. Cell Sci., **106(1)** 423-430 (1993). P.R. Mishra, S. Talegaonkar; Department Of Pharmaceutics, Faculty Of Pharmacy, Hamdard University, New-Delhi-6, India.

36. CONTROLLED RELEASE OF DICLOFENAC SODIUM USING MULTIPLE EMULSION BASED SYSTEMS.

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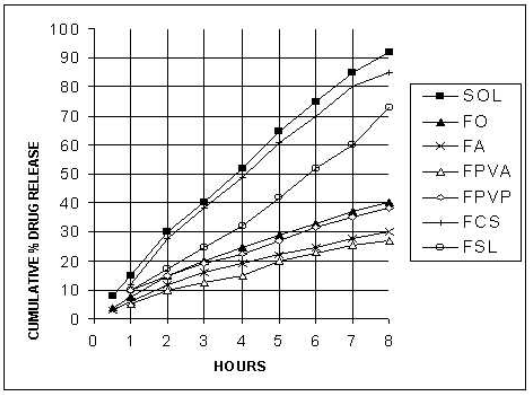
Purpose Multiple emulsions (W/O/W) are complex systems in which both types of emulsions (i.e. W/O and O/W) exist simultaneously and they require at least two stabilizing surfactants (1, 2). The migration of surfactants from one interface to other on formation of the completed multiple emulsion system is one cause of the multiple emulsion instability. A stabilizing film can be formed through the interfacial interaction between macromolecules in the internal aqueous phase and a lipophilic non-ionic surfactants in the oil phase (3, 4). The present investigations were aimed at exploring the potential of therapeutically acceptable multiple emulsion by attempting to stabilize it by interfacial complexation method. Diclofenac Sodium (DS), which is nonsteroidal anti-inflammatory drug, was used as model drug. Pharmacological and pharmacokinetic parameters of DS necessitate its controlled release preparation. **Methods** An improved version of the two-step emulsification procedure was employed (5) for preparing multiple emulsions (W/O/W). With sesame oil as middle organic phase, six formulations namely F_o (without macromolecule), F_A, F_{PVA}, F_{PVP} (with acacia, polyvinyl alcohol and polyvinyl pyrrolidone in internal aqueous phase), F_{CS} and F_{SL} (with calcium stearate and soya lecithin in middle oil phase) were prepared. The multiple emulsions (W/O/W) were characterized for their various physico-chemical attributes namely particle size (by a microscopic method), number of multiple oil droplets per mm^3 (by a Haemocytometer cell), partition coefficient of DS (by the shaking flask method) and release of the drug (by dialysis method). **Results** The mean number diameter of multiple oil droplets of formulation F_{PVA}, F_A and F_{PVP} were found to be 10.0, 12.0 and 18.0 μm respectively. There appeared to be no significant change with time in the drop count per mm^3 for the formulations containing macromolecules in the internal aqueous phase. The formulations with acacia and PVA in the internal aqueous phase were found to be relatively stable than those prepared using only span 60 (Fo) as an emulsifier. It may be seen from the plot of cumulative percent drug release Vs time that the release of the encapsulated drug from the W/O/W emulsion was slower compared to that of an aqueous solution used as reference. The release of DS was retarded significantly in case of formulations F_{PVA} and F_A as compared to release profile recorded for the formulation $F_{PVP.}$ From these studies, it is inferred that in the presence of acacia and PVA the partition coefficient of drug between oil and aqueous phases decreased, while no significant change was observed in presence of PVP (partition coefficient in $F_{o,}$ F_A, F_{PVA} & F_{PVP} was 1.75, 0.60, 0.55 & 1.70). Interestingly, the release rate of DS increased for formulations F_{CS} and F_{SL} (Partition coefficient 7.12 & 2.90 respectively).



Conclusion It is concluded from the study that multiple emulsions containing DS can be used for release rate modification. **Acknowledgement** The authors are thankful to CSIR, New Delhi for financial aid and Lekar Pharma Pvt.Ltd. India for providing the sample of Diclofenac Sodium. **References** (1) Frenkel, M., Schwart, R. and Garti, N., Stability: Inversion, apparent and weighed HLB. J. Colloid Interface Sci., 94, 174-178, 1983. (2) Magdassi, M., Frenkel, M., and Garti, N., Correlation between nature of emulsifier and multiple emulsion stability. Drug Dev.Ind. Pharmacy, 11, 791-798, 1985. (3) Law, T.K., Florence, A.T., and Whately, T.L., Stabilization of W/O/W multiple emulsion by interfacial complexation between macromolecules and non-ionic surfactants. Journal of Controlled Release, 3, 279-290, 1985. (4) Omotosho, J.A., Law, T.K., Whately, T.L. and Florence, A.T., Stabilization of W/O/W multiple emulsion by interfacial complexation between albumin and non-ionic surfactants. Colloid and Interfaces, 20, 133-144, 1986. (5) Florence, A.T. and Whitehill, D., The formulation and stability of multiple emulsion. International Journal of Pharmaceutics, 11, 277, 1982.

37. IN VITRO EVALUATION OF DRUG RELEASE FROM PROTEIN BIOFILM: APPLICATION TO LIDOCAINE.

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The therapeutic activity of an active ingredient after its administration is the combined result of numerous processes linked both to the active ingredient itself together with the patient's physiological variables, and to the biopharmaceutical characteristics of the dosage form in which the drug is administered. Numerous excipients now afford a regular controlled release of active ingredients for optimizing administration. Thanks to these excipients, sustained, time or controlled release systems are developed to supply the optimal concentration of a drug for a longer duration and with more efficiency. In most cases, the excipients used are polymers, natural and synthetic, with special properties, in particular gelling and swelling, which allow controlled release to be achieved. Such materials derive from various sources, the food industry being an important one. Among the different natural polymers, food proteins are very good candidates as functional excipients in the formulation of drug dosage forms. Apart from their nutritional properties, protein powders have functional properties such as dispersibility, swelling, adhesion, viscosity cohesive, film and fiber making that contribute to their major roles of color, texture and flavor in food systems. Pharmaceutical formulation can profit from the functional properties of food proteins by developing new controlled release drug dosage forms. Food proteins, especially soy and whey proteins possess several functional properties important for biofilm formation (1) Different pharmaceutical applications of biofilm as coating materials have been investigated such as aqueous pseudolatex of zein for film coating of solid dosage forms or gliadin films as a carrier for controlled drug delivery. (2). The aim of this work was to evaluate protein biofilm as drug dosage form for buccal or peroral sustained release administration using lidocaine as drug tracer. Whey proteins were used as protein source. Protein biofilm were prepared from betalactoglobulin with ethylene glycol as plasticizer. The protein/plasticizer ratio was equal to 1. After drying, a uniform film was obtained and stored (T:23°C and RH:43%) to await further use. The amount of lidocaine was 1,5 mg/cm^2. Development of protein biofilm with various thickness and different amount of protein was realized in order to investigate formulation variables on drug release. In vitro drug release was performed using the paddle method with dissolution medium of different pH. Lidocaine was essayed by a specific HPLC method. Milk proteins possess several functional properties important for biofilm formation. Most of the drug is entrapped in the network and do not remain at the surface of the biofilm as shown by the experiment realized with and without washing the film after its preparation. The results obtained exhibit that the release of lidocaine from the different biofilm formulated is delayed and influenced by pH. The release rate of drug is controlled by the protein amount and the thickness of the biofilm. 1 – V. Stella, P. Vallée, P. Albrecht and E. Postaire, Gliadin films : preparation and in vitro evaluation as a carrier for controlled drug release, Int. J. Pharm, 121, 117-121,1997g 2 – M. Subirade, I. Kelly, J. Gueguen and M.Pézolet, *Molecular basis of the film formation from a soy protein: comparison between the conformation of glycinin in aqueous solution and in film state*, Intern. J. Biologic. Macromol., 23 :241-249, 1998.

38. MATHEMATICAL MODELING OF VOLUME-SHIFT DURING QUALITY CONTROL AND DEVELOPMENT OF DISSOLUTION TEST PARAMETERS FOR PHARMACEUTICAL PRODUCT SOLID DOSAGE UNITS.

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Purpose: The dissolution rate of a solid pharmaceutical dosage unit is a critical parameter in the design of tablet or capsule product formulation. In most instances the progress of dissolution is monitored by sampling the dissolution medium at specified times for assay and the volumes withdrawn during each sampling can affect drug concentrations of subsequent sampling from the same dissolution medium. The USP 24 <720> monograph specifies that either the sample volume withdrawn should be replaced with an equal volume of fresh medium or the volume change should be taken into calculation of dissolution data. The account of changes in the dissolution medium volume is particularly important during dissolution profile studies of a rapid dissolution drug when sampling occurs frequently over a short time interval. **Methods:** This study, using USP dissolution standard tablets, describes the validation of a mathematical model designed to permit simple calculations of drug concentrations in the dissolution medium when sampling volumes are either replaced or not replaced following each sampling. **Results:** The applicability of this model is demonstrated by the extent of deviations in drug concentrations derived with and without volume correction. The extent of deviations was also observed to be a function of the sampling frequency and the number of samples withdrawn during dissolution profile studies. **Conclusion:** The mathematical model presented is applicable to the calculation of drug concentrations in the dissolution medium following replacing or not replacing the sampling volume with fresh medium.

39. MICROSCOPIC NMR IMAGING STUDIES OF CONTROLLED RELEASE DOSAGE FORMS IN AN APPROVED USP DISSOLUTION APPARATUS.

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PURPOSE. Demonstration of the use of ^{1}H NMR imaging to study physical changes occurring in controlled release devices during dissolution in an approved USP apparatus and comparison with total drug release monitored by UV-visible spectroscopy. **METHODS**. An USP apparatus IV *in-vitro* cell constructed entirely of plastic was housed in a commercial (Bruker Spectrospin) NMR imaging probe with a 30 mm diameter coil. The dissolution media were circulated at a controlled flow rate using a peristaltic pump (Gilson MiniPuls 5) from the reservoir (held at 37 °C) then through the USP apparatus which was maintained at a temperature of 37 °C. Series of ^{1}H NMR images through user-selectable planes within the tablets were obtained for several different types of controlled release devices, and the imaging parameters were optimized for various dissolution media. In some cases, the total drug release was also monitored as a function of time using UV-visible spectroscopy. **RESULTS**. A variety of controlled release devices including hydroxypropylmethylcellulose (HPMC) tablets, osmotic pump tablets, and compression-coated regulated release dosage forms were studied (one, 2). The NMR images of the tablets revealed the physical changes occurring in them during the dissolution process. In one case (2) close examination of the images revealed the reason for the abnormal release behaviour detected from monitoring of the total drug release via UV-vis spectroscopy. Subsequent modifications of the outer coating of the release device were tested and optimized in order to achieve the desired release profile whilst maintaining tablet integrity. **CONCLUSIONS**. Non-invasive and non-destructive NMR imaging was used to identify physical changes in different controlled release dosage forms undergoing dissolution in an USP IV dissolution apparatus. The kinds of NMR imaging experiments presented here will be extremely useful in quality control and will provide a very powerful technique capable of facilitating the development, modification, and improvement of controlled release devices. **REFERENCES**. (1) Fyfe, C.A; Grondey, H.; Blazek-Welsh, A.I.; Chopra, S.K.; Fahie, B.J. NMR imaging investigations of drug delivery devices using a flow-through USP dissolution device. *J. Control. Release*, 1:73-83, 2000. (2) Fahie, B.J.; Nangia, A.; Chopra, S.K.; Fyfe, C.A.; Grondey, H.; Blazek, A. Use of NMR imaging in the optimization of a compression-coated regulated release system. *J. Control. Release*, 51:179-184, 1998.

40. OPTIMIZING TWO-STEP DESOLVATION PROCESS FOR PREPARING GELATIN NANOPARTICLES.

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Purpose: Although the use of gelatin as a base for nanoparticles has been well documented, but success of the technique requires considering different parameters. In this work, we studied the effect of different parameters on the preparation of gelatin nanoparticles. **Method:** 1.25g gelatin was dissolved in 25ml distilled water at different temperatures (25, 40, 50, 60°C). 25 ml acetone was added to the gelatin solution as a desolvating agent, to precipitate the high molecular weight (HMW) gelatin. The supernatant solution was discarded and the HMW gelatin was dissolved by adding 25 ml distilled water and stirring at 600rpm at definite temperature. The pH of the gelatin solution was adjusted at 2.5 and after that 75 ml acetone added dropwise, at the end of the process 400ml of 25% glutaraldehyde solution as a cross-linking agent, and were left stirring for 12h. The prepared nanoparticles were purified by evaporating acetone and washing the nanoparticles with distilled water and centrifuging in 100000 rpm for 10 minutes (Air Fuge, Beckman). Different parameters were changed in preparing nanoparticles, such as temperature, pH, concentration of glutaraldehyde, type of desolvating agent and gelatin. **Results:** The results showed that pH 2.5 is the best pH for preparing nanoparticles. Increasing the pH causes the agglomeration of the gelatin particles. Increasing the concentration of glutaraldehyde to 400ml decreases the particle size, but after that point the particle size increases. As a desolvating agent ethyl alcohol and acetone was used but nanoparticles prepared with acetone were smaller. The temperature that used for preparation of nanoparticles had important effect on the particle size of nanoparticles. The smallest nanoparticles were prepared using gelatin B at 40°C. **Conclusion:** By changing different parameters in preparing gelatin nanoparticles, particle size will change. Using gelatin B at 40°C and acetone as desolvating agent in two step desolvation method smallest nanoparticles can be produced. This method is reproducible for preparing nanoparticles, Polydispersity index of prepared nanoparticels in size distribution curve is below 0.05.

41. QUANTITATIVE NMR IMAGING STUDIES OF HYDROXYPROPYLMETHYLCELLULOSE (HPMC) AS A SWELLING CONTROLLED DRUG DELIVERY DEVICE.

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Purpose: The objectives of this study were to use NMR imaging to monitor the swelling behaviour of drug-free HPMC tablets in order to obtain quantitatively reliable polymer concentrations during the swelling process to develop a reliable model. In addition, drug concentrations and release from swelling HPMC tablets were also monitored for two fluorine containing drugs. **Methods**: Static in-vitro one-dimensional swelling of HPMC tablets was investigated by NMR imaging. The setup of these 1D experiments limited the water penetration to a single face of a flat faced tablet and the subsequent swelling and drug diffusion in one direction. HPMC concentrations of a swelling tablet were determined using ^{1}H imaging by initially measuring the T_1 and T_2 relaxation times of reference samples of known HPMC concentrations. The drugs investigated were fluorinated compounds that could be independently detected using ^{19}F imaging. The implementation of a more efficient pulse sequence allowed for the three-dimensional swelling investigation of a drug-free HPMC tablet that permitted the water penetration and the subsequent swelling to occur in all directions. Unlike the 1D investigations, the 3D swelling tablet involved swelling in both vertical and radial directions. To monitor this, two orthogonal 'slices', which had a thickness of 1 mm through the tablet, were selected to be imaged. **Results**: The data from the 1D studies yielded 1D profiles that gave the drug and polymer concentrations as a function of distance at different times throughout the swelling. The profiles from T_2 were limited in that their representation of the higher polymer concentrations WAS semi-quantitative at BEST, WHICH was evident when mass balance calculations were carried out. However, measurements based on T_1 enabled higher HPMC concentrations to be measured quantitatively and mass balance calculations agreed after the maximum HPMC concentration dropped below 70%. The results from the 3D swelling of a drug free HPMC tablet were obtained as individual slices that showed both the vertical and the radial swelling directions. The swelling profiles in terms of HPMC concentration as a function of distance in the two dimensions were determined and were related to the 1D data. **Conclusion**: NMR imaging can be used to non-invasively provide quantitatively reliable information such as concentration profiles of both HPMC and drug during swelling in both one and three dimensions. The HPMC data can be used to develop an appropriate model for the swelling process.

42. CHARACTERIZATION OF A NOVEL METABOLIC PATHWAY OF DICLOFENAC: N-HYDROXYLATION.

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Diclofenac is an AINS drug that causes protein adducts and direct hepatotoxicity. The purpose of this study is to investigate a novel metabolic pathway known to generate reactive metabolites, N-hydroxylation, using a specific colorimetric method. Hepatic microsomes of rats, mice, rabbits and induced forms of these species were incubated at 37^0 C and pH 7, 4 in presence of a NADPH generating system and different concentrations of diclofenac. CYP 450 specific inhibitors were incubated as well to determine the isoenzyme(s) responsible for the metabolite formation. Chemically synthesised diclofenac-hydroxylamine was used as a reference on TLC separation of an ethereal extract of the incubation aliquot. Heme iron spin state was also determined using a spectrophotometric method. A final concentration of 1mM diclofenac and an incubation time of 15 minutes were found to be the more potent parameters to generate diclofenac-hydroxylamine at a final protein content of 2-3 mg/mL. Rabbits gave the more meaningful results: 7, 64 nmole/mg protein/minutes of hydroxylamine forming activity for control male rabbits, 8, 03 for 3-methylcholantrene induced males, 9, 04 for isosafrole induced males and 0 for female control rabbits. Sulfaphenazole was a potent inhibitor in S.D male rats. TLC separation of the metabolites did not show spots of hydroxylamines, suggesting the unstable property of hydroxylamines. A Soret peak at 411 nm is dependent of diclofenac concentration without incubation and cofactor, whereas a Soret peak at 422 nm is also diclofenac dependent in incubated hepatic microsomes. These results show that diclofenac is metabolized to a reactive metabolite, presumably a hydroxylamine, which may be responsible for the hepatotoxicity of the drug. ***Merck Company Foundation National Summer Student Research Program Abstract***

43. DEVELOPMENT OF A PH-SENSITIVE POLYMERIC MICELLE CARRIER SYSTEM FOR DOXORUBICIN.

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Purpose: pH-sensitive polymeric micelles (PM) consisting of N-isopropylacrylamide (NIPAM) copolymers were prepared and evaluated for the *in vitro* cell delivery of doxorubicin (DOX). **Methods:** Two different types of copolymers were synthesized by free-radical polymerization: 1) randomly-alkylated copolymers composed of NIPAM, methacrylic acid (MAA), octadecylacrylate (ODA) and *N*-vinyl-2-pyrrolidone (VP); 2) random copolymers composed of NIPAM, MAA and hydroxyethyl methacrylate poly(D,L-lactic acid) (HEMA-P(DLLA)). The copolymers were previously characterized with respect to phase transition pH, micelle size and critical association concentration (CAC). The anticancer drug DOX was incorporated into polymeric micelles by a dialysis procedure as follows: the copolymers and DOX were dissolved separately in N, N-dimethylacetamide (DMAc) or ethanol. In order to remove the hydrochloride salt groups from DOX, 1.3 molar equivalent of triethylamine was added to the DOX solution prior to mixing with the polymer solution. The resulting solutions were then dialyzed against water at 4°C for 24h. *In vitro* cytotoxic activity of polymeric micelles loaded with DOX, free DOX and unloaded micelles were assayed against C26 murine colon adenocarcinoma cells. Cell survival after 72 hours of incubation was monitored using the MTT assay. **Results:** The drug loading efficiency was higher using ethanol as solubilizing solvent for both polymers and DOX. However, DOX could be entrapped into poly ($NIPAM_{83}$-*co*-ODA_2-*co*-MAA_3-*co*-VP_{12}) (batch #1) and poly ($NIPAM_{95}$-*co*-ODA_2-*co*-MAA_3) (batch #2) micelles at concentrations reaching only 2% and 1.4% (*w/w*) respectively. Higher drug loading was achieved with P [NIPAM-*co*-MAA-*co*-HEMA-P (DLLA)] *i.e.* 5.1% (*w/w*) for monomer ratios of (83:5:1) (batch #3) and 4.5% (*w/w*) for monomer ratios of (83:5.5:0.5) (batch #4), with an entrapment efficiency of 17% and 15%, respectively. DOX entrapped in polymeric micelles batches 1 and 4 showed a higher cytotoxic activity against C26 cells (IC_{50} 347 and 243 nM respectively) than the free drug (IC_{50} 725 nM), while batch #3 was less cytotoxic (IC_{50} 1164 nM). The free copolymers generally showed low cell toxicity, although polymer #4 reduced the cell proliferation by 30%. **Conclusions:** DOX was entrapped with a modest yield into NIPAM-based polymeric micelles. However, based on the *in vitro* cell data, these micelles may represent a promising delivery system for poorly water-soluble anticancer drugs. **Acknowledgements:** This work was supported by the Natural Sciences and Engineering Research Council (NSERC) and by Labopharm Inc. (Laval, Canada). ***Merck Company Foundation National Summer Student Research Program Abstract***

44. EFFECT OF ST. JOHN'S WORT PREPARATIONS ON CELL VIABILITY, INDUCTION OF NITRIC OXIDE AND ETHOXYRESORUFIN O-DEETHYLASE ACTIVITY IN GLIAL CELL CULTURE.

***Marc Grenier*, Ping Sun Msc, Robert Drobitch PhD.; College of Pharmacy, Faculty of Health Professions, Dalhousie University, Halifax, N.S.**

Objective: To evaluate the effects of St. John's Wort preparations on cell viability, and the potential of such preparations to modulate induction of nitric oxide (NO) and cytochrome P4501A1/2-mediated ethoxyresorufin O-deethylase (EROD) activity in glial cell cultures. **Methods:** St. John's Wort (SJW) preparations, characterized for hyperforin, hypericin and pseudohypericin were provided by Dr. Brian Foster at Health Canada (Ottawa, Canada). Cells were isolated from the brains of newborn (<24hr) Sprague-Dawley rats and cultured in 20% FBS and Dulbecco's MEM until confluent (approximately 1 week). Cultures contained approximately 95% astrocytes and 5% microglia. Cells were exposed to SJW preparations (or media) + dibenzanthracene in serum-free media for 24hrs at SJW concentrations ranging from 0.005 to 2.0 mg/ml. At 24hr, aliquots were removed for measurement of lactate dehydrogenase (LDH) release (as a measure of cell viability) and nitrite levels (as a measure of NO production). Remaining media was then removed and replaced with fresh serum free media containing ethoxyresorufin and returned to incubation conditions. Resorufin concentrations were determined fluorometrically after a 3-hr incubation period. Finally, cell plates were scraped and homogenized for determination of protein and total LDH present in cell cultures.

SJW product	%hyperforin	%hypericin	%pseudohypericin
A (capsule)	4.195	0.080	0.133
B (tea bag)	0.122	0.020	0.034
C (tea bag)	0.670	0.018	0.038
D (capsule)	0.038	0.046	0.130

Results: Preparation A failed to induce NO over the concentration range studied. Preparation B produced NO levels peaking at 63 µM at 2mg/ml and preparation C peaked at 46 µM at 1mg/ml. Preparation D produced maximal NO levels of 16µM at concentration 0.25mg/ml. Preparation A failed to induce EROD activity in the cell cultures, potentially due to significant toxicity. Preparation C showed the largest production of resorufin, while preparations B and D showed minor induction of EROD activity. Both preparations A and D produced significant cell toxicity as witnessed by the percentage of LDH released vs. the total LDH present in the cell cultures. Preparations B and C were much less toxic, producing only half the % of LDH released vs. total LDH as preparations A and D. **Conclusion:** The SJW preparations studied were highly active in the model system studied. It appears as though SJW has the capacity to induce brain P450 enzymes, which may have implications for drug interactions. In addition, several SJW preparations showed high levels of NO. These levels could be cause for concern as other CNS diseases, namely Parkinson's, have shown that elevated NO levels correspond with CNS toxicity. **Acknowledgements:** Funding for this project was provided by a contract with Health Canada, the ***Merck Company Foundation National Summer Student Research Program*** and an Establishment grant from the Faculty of Health Professions.

45. EFFECTS OF A SYNTHETIC TRITERPENE ON THE REGULATION OF MATRIX METALLOPROTEASES AND TNF-α PRODUCTION BY MONOCYTIC CELLS FOLLOWING ENDOTOXIN, CYTOKINE OR CHEMOKINE ACTIVATION.

Melissa Skanes, **Michelle M. Fowler, Tram N.Q. Pham, Dennis D. Taub, James P. Kutney, and Vernon J. Richardson; School Of Pharmacy and Faculty Of Medicine, Health Sciences Centre, Memorial University Of Newfoundland, St. John's, Canada, A1B 3V6, National Institute For Aging, NIH, Bethesda Maryland, USA, and Department Of Chemistry, University Of BC, Canada.**

Purpose: The objective of this study was to determine if a synthetic triterpine (JPK-101) based upon compounds isolated from Triptertguim Wilfordii, a plant used in Chinese traditional medicine for the treatment of inflammatory diseases, could have any immuno-regulatory effects on the production of matrix-metalloproteinases (MMP) or inflammatory cytokines by activated monocytic cells. **Methods:** An in vitro model was used to assess the potential of JPK-101 as a possible new anti-inflammatory agent. MonoMac6 cells were stimulated with either endotoxin, TNF-α, TGF-β, MCP-1 or MIP-1β, or combinations of cytokine and chemokine. Production of MMP-2 and MMP-9 was assessed using gelatin-zymography and production of TNF-α was assessed using the L929 cell cytotoxicity assay. MMP-14 was assessed by indirect flow cytometry. Constitutive levels of TNF-α and MMP were determined in unstimulated cells as a base line. Other controls included the assessment of the effects of JPK-101 on the possible toxicity to MonoMac6 and L929 cells using a trypan blue exclusion assay. Sub-toxic doses of JPK-101 were used for the investigation of efficacy in this assay system. **Results:** JPK-101 was a very effective inhibitor of LPS induced TNF-α by MonoMac6 cells, with an ID50 of between 4-5 μg/ml. At six μg/ml, a total inhibition of TNF-α was observed. No toxicity was observed at concentrations of less than 12 μg/ml or less in MonoMac 6 cells and there was no observable cytoxicity or effects on the L929 bioassay up to 20 μg/ml. Preliminary data indicated that JPK-101 had no significant effects on the constitutive expression of MMP-2 or on LPS induced MMP-9, JPK-101 also had no significant effects on the TNF-α and TGF-β or chemokine regulation of MMP-2 or MMP-9 at 6 μg/ml. However, JPK-101 appeared to significantly inhibit the induction of membrane bound MMP-14 which has previously not been reported. **Conclusions:** JPK-101 appears to be a very effective inhibitor of LPS induced TNF-α by activated monocytic cells and may potentially be useful in treating certain inflammatory disorders such as rheumatoid arthritis where TNF-α is known to be actively involved in the disease pathogenesis. The lack of activity of JPK-101 against MMP-2 and MMP-9 production indicates that JPK-101 is ineffective in controlling these enzymes directly. It also indicates that the signaling pathways leading to TNF and MMP-2 and MMP-9 production are significantly different. The inhibitory effects of JPK-101 on MMP-14 appear to be novel and therefore require further investigation. If our observations are reproducible then JPK-101 may also be useful in the treatment of certain connective tissue diseases where MMP activation is a requirement. **Acknowledgements:** Funding for this project was provided by the Merck Foundation Summer Studentship (MS), PMAC Summer Studentship (MF), Faculty of Medicine and School of Graduate Studies Memorial University (TP) and the Faculty of Medicine Research and Development Fund. ***Merck Company Foundation National Summer Student Research Program.***

46. IN VIVO AND IN VITRO EFFECTS OF CYTOKINES AND BILE ACIDS ON HEPATIC EXPRESSION OF THE MURINE MRP2 MULTISPECIFIC ORGANIC ANION TRANSPORTER.

Annie Cheung, Georgy Hartmann and *Micheline Piquette-Miller*, PhD; Faculty of Pharmacy, University Of Toronto

Purpose. Experimental models of cholestasis in rodents are associated with dramatic reductions in the hepatic expression of the canalicular efflux transporter, *MRP2* (termed **cMoat**/ *ABCC2*). Hepatic cholestasis results in the release of the cytokines IL-6, IL-1β and TNFα as well as in the liver accumulation of bile acids. Pro-inflammatory cytokines *(Int. Immunopharmacol. 1:189-99; Mol Cell Biol. Res. Comm. 4: 248-56)* and bile acids have been previously demonstrated to alter the expression and transcription of several ABC transporters found in liver. In order to elucidate the mechanism(s) involved in *MRP2* downregulation, we examined the effect of bile acids and pro-inflammatory cytokines on the expression of *MRP2 in vitro* using a mouse hepatoma (Hepa 1-6) cell line and *in vivo* in mice administered cytokines or bile acids. **Methods.** Hepa1-6 Cells (n=4/group) were treated for 6 hr with 0, 25 or 100 µM of cholic acid (CA), taurocholate (TC) or taurodeoxycholate (TDC) or exposed to 0, 1 or 10ng/ml of TNFα, IL-6 or IL-1β. Male CD-1 mice (30±5g, n=3/group) were administered 1.5mM (ip) of CA, TC, or TDC or 10,000U (ip) of TNFα, IL-6 or IL-1β while controls received saline. Livers were collected after 24 hours. *MRP2* mRNA levels were measured in liver or Hepa1-6 cells by quantitative RT-PCR. **Results.** Both *in vitro* and *in vivo* studies demonstrated significant reductions in *MRP2* expression after cytokine treatments. *MRP2* mRNA levels were significantly decreased to 42±8%, 61±6% or 72±7% of control values in TNF, IL-6 and IL-1 treated Hepa1-6 cells ($p<0.05$), respectively. Likewise, *in vivo* doses of TNF, IL-6 or IL-1 resulted in significantly lower mRNA levels of *MRP2* (30-60% of controls). On the other hand, *in vivo* administration of bile acids did not significantly alter the hepatic expression of *MRP2*. Likewise, incubation of Hepa1-6 cells with CA and TC did not elicit significant effects, however TDC exposure resulted in a reduced *MRP2* mRNA expression (56±9% of controls, $p<0.05$). **Conclusions.** *In vivo* and *in vitro* findings suggest that the pro-inflammatory cytokines, TNF-α, IL-6, and/or IL-1β likely play an important role in *MRP2* downregulation during cholestasis. In comparison, our results indicate that in mice, the bile acids CA, TC & TDC likely play only a minor role in *MRP2* regulation. **Acknowledgements:** A. Cheung was a recipient of the ***Merck National Summer Student Research Scholarship***. Study funded by CIHR operating funds. *CIHR/ Rx&D HRF Research Career Award. Presented at the *American Society of Clinical Pharmacology and Therapeutics*, 103rd Annual Meeting, Invited Podium Session III-B, March 26, 2002, Atlanta, Georgia.

47. OXIDATIVE STRESS IN HEPATOCYTES: ROLE OF FATTY ACID BINDING PROTEIN.

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PURPOSE: Fatty acid binding protein (abbreviated FABP) is a cytosolic protein found in many tissues (e.g., liver, heart, intestine, epidermis, brain). FABP has been has been reported to be an integral intracellular protein for many metabolic reactions including the transportation of ligands through the cytosol and binding of metabolic byproducts, some of which are potentially lethal to cells and may include reactive oxygen intermediates. FABP represents about 4-5% of total intracellular proteins such that the cytosolic level is very high (»0.4mM). Because of its high intracellular concentration and the potential ability to bind oxidative products, FABP may play a key role as an endogenous free radical scavenger. Using dexamethasone treatment to reduce FABP levels, we tested the hypothesis that FABP exerts significant antioxidant activity in hepatocytes. **METHODS**: Rat (1548) hepatoma cells were incubated with (treated group) or without (control group) dexamethasone (0.5 µM) for three days at 37°C (95% O_2/5% CO_2). Following incubation, cells were loaded with 10 µM dichlorodihydrofluorescein diacetate (DCF). DCF was prepared in ethanol on a daily basis and kept at –20°C. The DCF solution was added to cells at room temperature in the dark. After 35 minutes of incubation, cells were washed with warm phosphate buffered saline (PBS) and incubated at 37°C for a further 2.5 min. This procedure was repeated twice to remove any external DCF. Oxidative stress was induced by incubating cells with 2 mM H_2O_2 for 8 minutes at 37°C. Following incubation, cells were washed twice with warm PBS and further incubated in PBS at 37°C for 2.5 min. Image analysis was started immediately following final incubation. Images were analyzed for fluorescence per cell area, which was taken as an index of intracellular reactive oxygen intermediates. RESULTS: Intracellular images from control and dexamethasone treated cells showed that incubation with dexamethasone resulted in a 290% increase in fluorescence per cell area (n= 161-256 cells, $p<0.01$). **CONCLUSION**: Dexamethasone treatment was associated with significantly higher levels of intracellular reactive oxygen intermediates. The decreased fluorescence in control cells likely resulted from the higher FABP levels. **ACKNOWLEDGEMENTS**: We gratefully acknowledge summer studentship support from the ***Merck Foundation*** for J. Bonnetta and CIHR (# MT 13683).

48. PHARMACOGENETICS OF CODEINE BIOACTIVATION IN PEDIATRIC DENTAL PATIENTS: DEVELOPMENT OF A REAL-TIME, RAPID-CYCLE PCR METHOD FOR CYP2D6*10 GENOTYPING.

Evan H. Kwong**, Thomas K. H. Chang, and Marc Levine; Faculty of Pharmaceutical Sciences, the University Of British Columbia, Vancouver, B.C.**

Purpose: The analgesic effect of codeine is due to its bioactivation to morphine by cytochrome P450 2D6 (CYP2D6). The overall aim of this study is to determine the effect of the CYP2D6*10 allele on the bioactivation of codeine to morphine in Asian children undergoing procedures at the dental clinic of Children's and Women's Health Centre of British Columbia. The purpose of our initial project is to develop a real-time, rapid-cycle polymerase chain reaction (PCR) method for genotyping CYP2D6*10. **Methods**: Based on a literature search of the CYP2D6 allele frequencies in Asians, we concluded that in order to accurately genotype 2D6*10 (C188→T), it will be necessary to rule out 2D6*5 (gene deletion) and 2D6*4 (C188→T, and G1934→A splicing defect), and to determine the presence of either 2D6*1 or 2D6*2 (C188). A primer set specific for 2D6 (F1/R1) and hybridization probes used for the 2D6*10 locus was designed and synthesized. A set of primers (P11/P12) used in a published 2D6*10 PCR-RFLP assay was also synthesized. This set amplifies both 2D6 and the 2D7BP pseudogene (the CYP2D locus contains four pseudogenes named 2D7P, 2D7AP, 2D7BP, and 2D8P). Single nucleotide polymorphisms (SNPs) were detected with the Lightcycler™, which uses the principle of fluorescence resonance energy transfer between two hybridization probes flanking the site of the SNP. In addition, we performed PCR-RFLP analysis of the 2D6 exon 1 amplicon with the restriction enzyme *Hph*I to validate the results. **Results**: Rapid-cycle PCR and melting curve results for 2D6*10 with F1/R1 primers were inconsistent. Although consistent results were obtained with the P11/P12 primers, these primers also amplified the 2D7BP pseudogene, which has the C188→T mutation. In this case, two peaks were seen: one at 59°C (2D7BP – C188→T) and another one at 68°C (2D6 "wildtype"). Because consistent results were obtained with P11/P12 primers, but not with the F1/R1 primers, we conducted experiments with the P11/R1 primers for the 2D6*10 analysis (R1 is specific for 2D6). The initial rapid-cycle PCR and melting curve results showed good reproducibility and specificity for 2D6, with a single peak at 68°C. Subsequent PCR-RFLP analysis of 2D6*10 produced results that are in agreement with the melting curve analysis. **Conclusions**: The 2D6*10 C188→T SNP can be detected by real-time, rapid-cycle PCR. Our novel approach for genotyping CYP2D6*10 in patient samples will involve initial analysis for the *5 gene deletion by long chain PCR, followed by subsequent analysis for the *10, *4, *1, and *2 alleles by real-time, rapid-cycle PCR. **Acknowledgements**: This research was supported in part by the B.C. Children's Hospital Telethon Fund and the Dawson Endowment Fund. E.H.K. received a ***Merck Company Foundation National Summer Studentship***. T.K.H.C. is the recipient of a Research Career Award in the Health Sciences from CIHR and Rx&D Health Research Foundation.

49. TOWARDS THE SYNTHESIS OF SYMMETRICALLY GALACTOSYLATED β-CYCLODEXTRIN DERIVATIVES.

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Purpose: Gene therapy has spurred active research in the area of novel delivery techniques. Polymer-based synthetic gene delivery vectors with potentially minimal immunological activity are gaining support as benign transfection agents. Additional advantageous features of these non-viral vectors include: (a) the ability to introduce DNA into non-dividing cells, (b) attenuated capacity for integration into chromosomes, and (c) potentially reduced production costs compared to the scale-up production of viral vectors. Moreover, the incorporation of specific targeting ligands into these polymer delivery systems has led to synthetic vectors that display tissue tropism. The objective of our work was to prepare a symmetrically galactosylated β-cyclodextrin (β-CyD) with the intent of conferring hepatocyte tropism. Once synthesized, this modified β-CyD may potentially serve as a suitable synthetic vector for hepatocyte-targeted drug delivery. In addition, the ability of β-CyDs to form inclusion complexes with aromatic rings could potentially be exploited to prepare "coated" oligonucleotides, which may target hepatocyte asialoglycoprotein receptors. **Methods (general)**: Reactions were generally carried out in dry solvents under argon atmosphere. Reactions were monitored by thin layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) and visualized by charring with methanolic sulphuric acid spray. E. Merck silica gel 9358 (60, particle size 0.040 – 0.063) was used for flash column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM300 spectrometer using residual non-deuterated solvent as internal reference. **Synthesis**: The strategy for the assembly of heptagalactosyl-β-CyD involved the preparation of two components, namely: (a) a hepta-amino-CyD derivative, and (b) a galactose derivative armed with a linking arm for conjugation to the CyD carrier. Preparation of the first component involved the symmetrical modification of β-CyD. Thus, direct halogenation of unprotected β-cyclodextrin provided the hepta-6-bromo-6-deoxy-β-CyD as per a literature protocol. Subsequent halogen displacement with azide then provided the hepta-6-azido-6-deoxy-β-CyD. This azido compound was acetylated for convenient purification by flash silica gel chromatography. Following purification, the blocked β-CyD was de-O-acetylated to provide pure hepta-6-azido-6-deoxy-β-CyD and then methylated to yield the hepta-6-azido-6-deoxy-2,3-di-O-methyl-β-CyD derivative. Finally, the 6-azido moiety was reduced to provide the required hepta-6-amino-6-deoxy-2,3-di-O-methyl-β-CyD component. These reactions followed literature protocols with minor modification, and were readily performed on the multigram scale. All spectra were consistent with the proposed structures and with previously published data. The second component involved the assembly of a galactose derivative equipped with a linking arm suited for subsequent conjugation to the amino-CyD scaffold. We envisioned the preparation of a galactose derivative with armed with an azido-substituted diethylene glycol aglycon as a suitable intermediate that could readily be activated for coupling to the amino-CyD component. The linking arm was derived from a sequence beginning with diethylene glycol (DEG). Thus, DEG was tosylated, and the resulting monotosyl-DEG product isolated. The tosyl group was subsequently displaced with azide to provide the required linking arm aglycon. Reaction of this aglycon with acetobromo-α-D-galactose in the presence of silver salts provided the corresponding β-galactoside in 65% yield. This galactose derivative can be activated for coupling via a routine three-step protocol leading to an activate ester. Conjugation of the activated galactose derivative to the amino-CyD carrier proceeded smoothly to provide the target heptagalactosyl-β-CyD (65% yield) upon subsequent deprotection. **Results**: Preparation of the key hepta-6-amino-6-deoxy-2,3-di-O-methyl-β-CyD was accomplished on the multigram scale. Additionally, the reaction of galactose with an appropriate linking arm has provided the key galactose precursor. Final activation and conjugation of this galactose derivative to the hepta-amino-CyD has since been carried out to provide the symmetrically galactosylated β-CyD. **Conclusions**: The strategy for the assembly of ligand-armed cyclodextrins, described herein, has led to the preparation of an hepta-galactosylated-CyD in a manner suitable for scale-up. The ability of this β-CyD carrier to selectively target the hepatocyte asialoglycoprotein receptor awaits further evaluation. **Acknowledgements**: This work was supported by the ***Merck Company Foundation National Summer Student Research Program*** under sponsorship from the University of British Columbia, and in part through CIHR grant #13480. R.S. also wishes to acknowledge Dr. Kishor M. Wasan, University of British Columbia, for his assistance.

50. VALPROIC ACID INDUCES DOWNREGULATION OF HOXA2 GENE IN THE DEVELOPING MURINE PALATE.

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Hoxa2 gene is a member of the clustered homeobox gene family that plays an important role in the genetic control of embryonic develoment. Mice homozygous for a targeted mutation of the Hoxa2 gene are born with a cleft-palate and multiple cranial abnormalities. **Purpose**: (1). To determine the temporal and spatial expression profile of the Hoxa2 mRNA and protein in the developing murine palate. (2). To determine if valproic acid, a clinically used anticonvulsant drug and a known teratogen (which induces birth defects including cleft-palate) alters Hoxa2 gene expression profile in the developing palate. **Methods:** Mouse embryos were staged according to Kaufman (1992). The morning on which a vaginal plug was found was considered day 0 of pregnancy. Embryos (E12.5 to E15) dissected from timed-pregnant Swiss-CD-1 mice were fixed overnight in 4% paraformaldehyde in PBS, after which the embryos were rinsed three times in 20% sucrose in PBS and cryoprotected overnight in fresh 20% sucrose solution. Frozen embryos in embedding medium were cryostat sectioned (8μm thick) and tissue sections collected on gelatin-coated coverslips. The coverslips were left to dry at room temperature to between 2h and overnight before being subjected to immunohistochemical analysis. The primary antibody used was anti-Hoxa2 polyclonal antibody (1:1000) and the secondary antibody utilized was biotinylated goat anti-rabbit IgG (1:500). For in-situ hybridization histochemistry, a 518-basepair segment of the Hoxa2 gene from the nucleotide residues 681-2199 was subcloned into pBluescript II SK+ and used as template for the synthesis of digoxigenen-labelled RNA probes. For the drug treatment studies, sodium valproate was administered subcutaneously in three doses to timed-pregnant mice, 400mg s.c. at E8 and E10; 600mg s.c. at E12.5. Control embryos were adminstered saline. Treated and control embryos were dissected out at E13.5 and E15 and subjected to immunohsitochemical analysis and in-situ hybridization histochemistry. **Results**: At the embryonic stages E12 to E12.5 when the palatal shelves are just forming, Hoxa2 gene expression is mostly expressed in the palatal epithelium. As the palatal shelves become vertically directed at stages E13 and E13.5, Hoxa2 gene is strongly but diffusively expressed in the palatal epithelium and mesenchyme. At E14, the palatal shelves elevate and become horizontally directed and Hoxa2 expression is downregulated and restricted mainly to the palatal epithelium. As the two adjoining palatal shelves begin to fuse at E14.5, strong expression is visible in the epithelial seam. By E15 when the shelves have completely fused down regulation of Hoxa2 is evident. Valproate treated palatal shelves were remarkably devoid of Hoxa2 mRNA and protein expression. Lower Hoxa2 expression profile correlated with a delay in palatal fusion. **Conclusions**: Temporal and spatial Hoxa2 expression profile in the developing palatal shelves implicates a role for Hoxa2 gene in palatogenesis. Hoxa2 gene expression is down regulated in palatal shelves after treatment with sodium valproate that correlates with the development of a cleft-palate. **Acknowledgements**: Funding for this project was provided by the Natural Sciences and Engineering Research Council of Canada to AN and from the ***Merck Frosst Company*** in the form of summer studentship to RO.

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Richard	Smith	Agilent Technologies	Canada
Victor	So	Postdoctoral Fellow, University of Toronto	Canada
Bob	Sparks	Particle and Coating Technologies Inc.	USA
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Nikolay	Stoynov	BRI Biopharmaceutical Research Inc.	Canada
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Jonathan	Willmer	CroMedica Prime Inc.	Canada
Glenn	Wong	University of British Columbia	Canada
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Al	Yergey	National Institute of Child Health	USA
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The speakers, posters, chairs

CSPS Symposium Survey

Please take a moment to complete the following survey.
Tear it out and turn it in to the Registration Desk. Thanks.

1. What location would you prefer for the next CSPS conference in 2003?

2. Suggestions re theme for next years conference.

3. Comments re this years conference.

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To apply, complete this form and submit it by faxing to 1 780 492 0951, or mailing to CSPS, 3118 Dentistry/Pharmacy Centre, University of Alberta Campus, Edmonton, Alberta, Canada, T6G 2N8. Payment may be made by VISA, cheque or money order. If paying by VISA, please be sure to include your credit card number and expiry date. (Note: Memberships expire at the end of the calendar year).

email: Sandra.Hutt@ualberta.ca
http://www.ualberta.ca/~csps

Membership Categories

Individual: This membership is comprised of international scientists involved in various stages of pharmaceutical research and development.
Fee: $100.00

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CSPS

The Canadian Society for Pharmaceutical Sciences (CSPS), is a non-profit organization which was established in 1996 to foster excellence in pharmaceutical research. CSPS membership includes scientists world-wide, who are involved in all aspects of pharmaceutical sciences with affiliations ranging from academia, industry to government. The electronic "Journal of Pharmacy and Pharmaceutical Sciences" is the official, international journal of CSPS.

Society Mission

The mission of the Canadian Society of Pharmaceutical Sciences is to establish a network of pharmaceutical scientists in Canada, and make contact with sister national/international agencies in pursuit of excellence in research, development, and training. The mission includes an electronic journal, the "Journal of Pharmacy & Pharmaceutical Sciences".

The specific goals of the CSPS are:

- To advocate excellence in pharmaceutical research
- To promote allocation of funds for pharmaceutical research
- To be involved in decision and policy making processes at the government level
- To establish an international peer-reviewed electronic journal dedicated to publication of pharmaceutical findings
- To encourage the continued development of an improved environment for industry-based pharmaceutical research in Canada

The Society fully supports the goals and objectives of the Association of Faculties of Pharmacy of Canada (AFPC) but encompasses a larger population of Canadian pharmaceutical scientists from not only academic institutions, but also governments, and the private sector.

Society Profile

Over the past several years, pharmaceutical research in Canada has undergone considerable changes. For example, in addition to the activities in the pharmacy schools and established pharmaceutical houses::

- It has expanded to academic units other than faculties of pharmacy
- Many small but dynamic research and development and contract houses have emerged
- The public funding for research has been substantially reduced.

In light of these changes, it was found timely to assemble all those individuals characterized as Canadian pharmaceutical scientists under one organization, the Canadian Society for Pharmaceutical Sciences (CSPS).

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- Comparability and equivalence challenges of biologics and biotechnology derived therapies and other complex drugs.
- Imaging techniques used in drug discovery and development.
- Student presentations.

Conference proceedings will be published in the Journal of Pharmacy & Pharmaceutical Sciences.