Exploring Human Diversity in Drug Development & Pharmacotherapy

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Drug Development & Pharmacotherapy

Invited Speakers

Pharmaceutical sciences in the next milennium.

D.J. Triggle, State University of New York, Buffalo

Twelve technologies are predicted to be critical to our forthcoming competitiveness. Included in the Life Sciences are "bioprocessing", "drug design" and "targeted pharmaceuticals".

Frank Press, National Academy of Sciences, USA, 1992.

Despite the therapeutic successes of the past fifty years, there has never been a more urgent need for the development of new therapeutic agents – from emerging viruses, to antibiotic-resistant bacteria, third-world pandemics, population control and the protection of food sources – there is an increasing imperative for new agents and methodologies. Compare this to the optimistic words of Paul Ehrlich almost a century ago:

"Now that the liability to, and danger of, disease are to a large extent circumscribed – the effects of chemotherapeutics are directed as far as possible to fill up the gaps left in this ring".

Paul Ehrlich, 1913

The "new" pharmaceutical sciences will be dominated by the paradigms of molecular biology. But this will not be merely a 21st Century Therapy Mall where patients shop for designer jeans and designer genes, but rather an all-encompassing translation of molecular biology into chemistry, including combinatorial chemistry and self-reproducing molecules, to drug screening and delivery mechanisms to patient profiling and individualized therapeutics. Increasingly, therapeutics will move from phenotypic to genotypic treatment with both the ascendancy of pharmacogenomics and the economic impact of the increasing cost of health care delivery.

These aspects of the continuing development of pharmaceutical sciences and therapeutic delivery will be discussed and illustrated in terms of the predicted developments in basic sciences and therapeutics.

David J. Triggle, Pharmaceutical Sciences in the Next Millennium, The Annals of Pharmacotherapy 33: 241, 1999.

New advances in human diversity. Pediatric issues in pharmacokinetics and pharmacodynamics.

J. S. Leeder, The Children's Mercy Hospital, Kansas City, Missouri

The goal of rational drug therapy is to produce a desired pharmacological response in an acceptable and predictable manner while minimizing the occurrence of undesired events. At present, there is significant interest in understanding how pharmacogenetics and pharmacogenomics may contribute to our understanding of interindividual variability in the clinical responses to therapeutic agents. In the context of pediatric pharmacotherapy, genetic and environmental determinants of variability are superimposed on a changing background of development and maturation to add further complexity to optimal medication use.

For many years, accepted dogma suggested that drug biotransformation capability was limited at best in the fetus and newborn but increased over the first year of life to levels in toddlers and young children that generally exceeded adult capacity. In fact, there are several situations where examination of clinical pharmacokinetic data has revealed discernable patterns of drug clearance that can be attributed to developmental differences in drug biotransformation. As knowledge of mammalian drug biotransformation processes has increased over the past few years, it has become apparent that not only are there developmental differences in expression among drug metabolizing enzyme families (CYPs, glucuronosyl transferases etc.), but that individual drug metabolizing enzymes may have unique developmental profiles which in turn influence the therapeutic response, desired or undesired, to a given agent. The unique developmental patterns of individual CYP isoforms will be illustrated using published *in vitro* and *in vivo* data as well as preliminary data from a phenotyping study currently in progress. Since altered drug biotransformation significantly impacts drug efficacy and toxicity, characterizing the ontogeny of these pathways during development is essential for developing optimum treatment strategies, especially in the first year of life.

Age issues in pharmacokinetics and pharmacodynamics.

D.S. Sitar, University of Manitoba, Winnipeg

It is now widely appreciated that the elderly cohort in our society is growing more rapidly than any other age group. In fact, it is predicted that the largest increase in population will occur in those persons older than 85 years. Yet until very recently, this segment of the population was virtually ignored with respect to drug development and pharmacotherapy. There have been many generalizations concerning the disposition and efficacy of drug therapy in older persons. Most often, these generalizations have evolved based on cross-sectional data that did not carefully control for the presence of multiple and chronic diseases that occur more often in this cohort. This presentation will focus on evidence of age effects on drug disposition and effect.

There is good evidence that body composition changes with increasing age. Based on the physicochemical properties of drug substances, it is predictable that apparent volume of distribution will be affected by changes in body composition. It is important to recall that a change in apparent volume of distribution will alter the half-life of a drug in the circulation in the absence of a decrement in the person's ability to elimi-

nate it from the body. Therefore reliance on half-life in isolation as an indicator of ability to eliminate a drug will confound attempts to individualize drug therapy.

With respect to organ function, liver size decreases as age increases. Depending on the extraction ratio for a drug, a dose may overwhelm the ability of a smaller organ to limit peripheral drug concentrations. This effect can be predicted to make drug therapy more difficult in the elderly, but suggests also that the choice of drug is an important factor in minimizing this problem. As well, circulating albumin concentration decreases in the elderly even in the absence of clinical disease. Thus, drugs used in high doses that are extensively bound to albumin can be predicted to be more likely to cause toxicity when therapy is based solely on total drug concentration. Kinetic changes in pulmonary gas exchange occur with increasing age. Thus the use of end tidal anesthetic concentrations during surgery as an indicator of depth of anesthesia is likely to be flawed.

Examples of pharmacodynamic changes with age are more difficult to evaluate because of the pharmacokinetic changes with age presented above. Although a pharmacodynamic explanation is offered for the observed increased sensitivity of the elderly to central nervous system depressant drugs, this interpretation is controversial. Evidence is more convincing for reduced pharmacodynamic response to b-adrenoreceptor agonists in the elderly, and has implications for the use of b-blocker drug treatment in them. Yet definitive studies in this patient population to test this hypothesis remain to be completed.

In summary, there is reasonable evidence for important changes in both pharmacokinetic and pharmacodynamic parameters with increasing age, even in the absence of disease. These differences have important implications for the choice of study subjects in order to optimize drug therapy for that cohort of society that is most reliant on this approach to disease management.

Influence of diseases on pharmacokinetics and pharmacodynamics: Multiple disease conditions.

F. Jamali, University of Alberta, Edmonton, Canada

The principle of pharmacotherapy is based on the assumption that the response by the general patient population to a pharmacological intervention is reasonably uniform within a range of dose or drug concentration. Hence, therapeutic recommendations are made based on therapeutic 'dosage range'. The problem with this approach is the inter-subject variability due to factors such as age, sex, race and, of course, pathophysiological conditions. For example pharmacokinetics and hence pharmacodynamics of oral analgesics are different in subjects with sever pain as compared with those with mild pain. With the aging population the possibility of multiple diseases increases. However, the data on disease-drug interactions usually concentrate only on the disease that the drug is indicated for. Hence, the effects of other diseases are often ignored. For example, heart diseases and stroke are number one killers in many societies. In addition over 11% of the world general population suffers from inflammatory diseases such as arthritis. The incidence of inflammatory diseases increases substantially with age. Interestingly, it has been reported that the control of blood pressure is problematic in the presence of arthritis. Further, very recently, treatment failure following myocardial infarction is attributed to inflammatory conditions.

Inflammatory conditions or the inflammation-induced pathophisiological changes may inhibit clearance of drugs by 1) reducing the unbound plasma drug concentration secondary to increased acute phase plasma proteins, and 2) reducing efficiency to metabolize drugs. The most likely candidates for such a disease-drug interaction is many of the cardiovascular drugs that are highly bound to a_1-acid glycoproteins and are efficiently metabolized by the liver, e.g., propranolol, verapamil. Indeed, elevated plasma concentrations of these drugs have been reported. Since pharmacokinetics is accepted as a surrogate marker of pharmacodynamics, an increased concentration of cardiovascular drugs, secondary to decreased clearance, may suggest increased efficacy or toxicity in arthritic patients. More recent data, however, suggest that quite contrary to general expectation, inflammation not only inhibit metabolism, it also down-regulate cardiovascular receptors such as those involved in the action of calcium channels and b-adrenoreceptor blockers; i.e., reduced effect despite substantial increase in plasma drug concentration. This is likely due to an over-production of inflammatory cytokines and/or nitric oxide, and suggests that a pharmacokinetic observation may not necessarily reflect pharmacodynamic outcomes. Since cytokines (e.g., various interleukins, tumor necrosis factors and interferon) may be used as drugs, the observation described here may also be extrapolated to drug-drug interactions in addition to drug-disease interactions. Altered cytokines expression which is reported to occur in many pathophiological conditions (e.g., inflammatory diseases, infection, asthma, aging) is likely to contribute to inter-subject variability in both pharmacokientic and pharmacodynamic observations. Factors contributing to inter-subject variability must be considered in designing clinical trials.

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Impact of pharmacogenetics on drug response.

E.M. Sellers, R.F. Tyndale, Departments of Pharmacology, Medicine and Psychiatry and Centre for Research in Women's Health, University of Toronto; Sunnybrook and Women's College Health Science Centre-Women's College Campus; and Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Purpose. Pharmacogenetic variations in the patterns of metabolism among individuals can importantly modulate the risk of adverse drug events including drug dependence. Concern is commonly directed towards safety and drug interaction issues. Cytochrome P450 drug metabolizing enzymes (CYPs), can "activate" (e.g. codeine to morphine) or "deactivate" (e.g. nicotine to cotinine) drugs of abuse. Some CYPs are polymorphic, that is, there are gene mutations which result in no active enzyme (null mutations). Individuals with two null mutations appear in the population as phenotypic "poor metabolizers". The presence of these mutations appears to have no impact on the health of individuals. Methods & Results. Using *in vitro* studies, we have identified drugs of abuse that are substrates of the polymorphic enzymes CYP2D6 (codeine, oxycodone, hydrocodone, amphetamines, dextromethorphan), CYP2A6 (nicotine) and CYP2C19 (diazepam, flunitrazepam, tetrazepam, carisopodol). In human experimental studies, we have shown that CYP phenotype and genotype affect abuse liability for CYP2D6 metabolized drugs of abuse (codeine, methamphetamine, dextromethorphan). In addition, we inhibited CYP2D6 and experimentally decreased individuals' risk of dependence (codeine, dextromethorphan). CYP2D6 inhibition with fluoxetine was also used to treat codeine dependence. In epidemiologic studies CYP2D6 and CYP2A6 null mutations protect individuals from becoming codeine and tobacco dependent, respectively. Conclusions. With respect to CYP2A6, heterozygote individuals, if they become smokers, smoke about 25% fewer cigarettes because of their slower nicotine metabolism. The resulting decrease in nicotine clearance decreases the intensity of smoking (and smoke exposure) thereby decreasing the risk of tobacco-related diseases. In addition, by inhibiting CYP2A6, smoking can be decreased in tobacco-dependent individuals. Since normally occurring mutations in CYP alleles decrease the risk of dependence, pharmacologic modification of CYP activity has the potential to prevent and treat drug dependence. These observations demonstrate that pharmacogenetic variations are not only important as determinants of drug safety but also by mimicking these defects that new approaches to treatment are possible.

Designing a pharmacogenomic experiment.

D. A. Katz, Abbott Laboratories, Abbott Park, Illinois

There is significant interest in the new field of pharmacogenomics (PG) - studying the role of genes in interindividual variation of drug response. The central questions are whether there is a genetic component for a particular drug response, and if so, which of our approximately 100,000 genes are related to an individual's reaction to that drug.

Using examples from our ongoing work with the leukotriene synthesis inhibitors Zyflo® and ABT-761, this presentation will:

- demonstrate that genes are involved in continuous traits such as many drug responses,
- introduce the methods we use to discover novel drug response-associated genes,
- provide guidelines for the number and type of samples needed to perform PG studies,
- describe the importance of placebo or usual care data as a comparator when selecting patients for PG studies.

Pharmacokinetics and pharmacodynamics and genotyping.

J. Steve Leeder, Children's Mercy Hospital, Kansas City, Missouri

Proteomics in drug discovery.

A. Ducret, Merck Frosst Canada & Co., Montreal, Quebec

The development of potent and specific drugs represents a complex process whose scope involves many different disciplines. To streamline the discovery and the validation of new compounds, pharmaceutical companies have typically relied on chemistry, for the synthesis of large numbers of structurally-varied molecules, and on high-throughput screening, for the biological testing of leads susceptible to contain potential candidates. Compounds of interest are then tested in whole cell assays and in animal models to investigate their pharmacological profile before being examined in humans. As a result, pharmaceutical companies have marketed far more drugs during the last five years than in the 1980's. This strategy, however, is performed at an enormous cost as 17 of 20 compounds submitted to Phase I fail through clinical trials.

Proteomics represents a relatively new discipline aiming at investigating biological systems globally and systematically at the protein level. As most serious illness is associated with imbalances among or malfunctions of proteins, proteomics al-

ready plays a major role for the discovery and validation of new therapeutic targets. Concomitantly, because drugs typically act at the protein level, global analysis can help unravel mechanisms of action that differentiate the unwanted side effects from the therapeutic treatment. In particular, proteomics appears promising for the characterization of knockout animals or cell lines that were genetically modified to lack the therapeutic target of interest. Thus, using organisms of identical genetic background, proteomics can assist in selecting drugs that minimize unspecific or undesirable protein interactions. While proteomics has represented a major breakthrough for the identification of new targets in the diagnostics area, its use in drug discovery has been hampered by the challenge of comprehensively and reproducibly analyzing proteins in a general fashion. This presentation will focus on the technological development allowing the general investigation of proteins and describe strategies that are currently developed at Merck Frosst to assist drug discovery.

Bioinformatics and drug discovery.

D. S. Wishart, University of Alberta, Faculty of Pharmacy & Pharmaceutical Sciences, Edmonton, Alberta

Advances in large scale DNA sequencing, rapid developments in high-throughput macromolecular structure determination and improvements in high-throughput chemical synthesis are fundamentally going to change the way drug discovery will be done in the future. At one level, these technological advancements will offer a tremendous opportunity to identify new targets and explore new vistas in rational drug discovery and development. At another level, these high-throughput techniques are going to provide such an avalanche of scientific information that we may not have the means or the methods to handle it. Indeed, it is projected that over the next 10 years, terabytes (trillions of bytes) of biological, structural and chemical data will be deposited in hundreds of publicly (and privately) held databases. How do we expect to deal with all of this information? One answer is bioinformatics. Bioinformatics is an emerging field of information technology which is attempting to develop the hardware and software tools needed to archive, link and "mine" this wealth of data. In this lecture I will attempt to give a broad overview of what bioinformatics is and how the tools that bioinformaticians are developing could potentially have and impact on both drug target and protein drug identification. I will also show how many newly emerging software tools will have a significant role to play in the field of pharmacogenomics and proteomics. Examples will be taken from work in my own laboratory, my industrial collaborators and from the recent literature.

Satellite Open Forum: Are the pharmaceutical sciences recognized in the new MRC/CIHR proposal?

F. Abbott, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C.. M. Bureau, Member, Interim Governing Council for CIHR; President, Fonds de la recherche en sante du Quebec.

Subject selection in drug testing: general overview.

J. Turgeon, Phoenix International Life Sciences, Montreal, Quebec

Historically, common sense and day-to-day experience have thought us that each individual is unique. Genomic studies have provided proofs to that. But now, we are discovering that individuals with the same disease may have it caused by different genes or different mutations within this gene. The Long QT Syndrome is an excellent example of this observation. As well, specific genotypes of candidate genes in the renin-angiotensin system are associated with an increased risk of in-stent restenosis. Consequently, it is expected that genetic science will fundamentally change the way in which drugs are discovered and developed. It is believed that in the future, the industry will retreat from developing broad-spectrum drugs and advance the development of medicinal agents for genetically defined subpopulations. This statement is supported by the growing number of reports showing marked differences in drug response among people of different makeup. One of the very good examples is the response to HMG-CoA reductase inhibitors and patient's genotype for the cholesteryl ester transfer protein. As well, in Alzheimer's disease, differences in apolipoprotein E (APOE) genotype appear to explain differences in response to drug treatment.

In the recent years, we have also learned that the disposition of drugs can differ from one individual to another. Polymorphisms in drug metabolic pathways have been characterized and very nice examples have been described for the cytochrome P450 superfamily. Knowledge of patient's phenotype or genotype may help prevent drug side-effects and even drug-drug interactions in specific subpopulations. In addition, patient's phenotype could be manipulated to increase drug efficacy or decrease toxicity. This is illustrated by recent data indicating increased efficacy of a Class I antiarrhythmic agent during chronic inhibition of CYP2D6 activity.

Finally, pharmacokinetic studies in well characterized subpopulations may help assess true variability associated with different formulations of the same drug. These types of studies must be well designed since they may also falsely decrease or

increase intra-subject variability for drugs subjected to saturable metabolism. Examples of drug tested for NDA applications will be discussed to illustrate how subject selection in drug testing could become a major advantage.

Inter-subject and intra-subject variability in clinical drug trials.

R.L. Lalonde, Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan

Diversity in a species is good for promoting survival of fittest individuals. However, variability between and within individuals complicates the design of clinical drug trials. Inter-subject variability will have an impact on the sample size for typical parallel trials used in Phase II/III while intra-subject variability will affect the sample size for typical crossover studies used for many Phase 1 studies. Data from identical/fraternal twins will be reviewed to contrast genetic and environmental causes of variability. More recent pharmacogenetic examples will be used to illustrate variability in pharmacokinetics and pharmacodynamics and how this information is being used in clinical trials. Intra - and inter-subject variability for typical pharmacokinetic parameters will be presented for a wide range of drugs. The focus of the presentation will be on the determinants of variability in the dose-concentration-effect relationship and how to manage this variability in drug development.

Subject selection in bioequivalence studies: Impact on highly variable drugs.

Y. Chung Tsang, Radu Pop and Michael Spino. Apotex Inc., Weston, Ontario, Canada

Bioequivalence (BE) is a very important element in the registration of generic drugs. It is also required when the brands change their formulation from the one used in clinical trials to the final marketed form or when they make important formulation changes at any time. Demonstration of BE between two products or formulations of an uncomplicated drug requires the ratio of means for AUC and Cmax be within ±20% of each other in a single-dose crossover BE study. In addition, the 90% confidence interval (CI) of the ratio has to be within 80 to 125%, based on log-transformed data. Since the width of the 90% CI depends not only on the difference in mean between products or formulations but also on the variability of the data, it is important that non-formulation related sources of variability be controlled in BE studies so that observed differences between test and reference products are reflective of formulation differences, not other unrelated variables. Failure to employ adequate control could result in a significant increase in number of subjects required to meet the 90% CI requirement for two products that are completely bioequivalent. This is especially important with highly variable drugs (intra-subject cv >30%).

A host of factors are known to contribute to variability in drug absorption and/or disposition between subjects. With the use of a crossover design in BE studies; each subject serves as his/her own control, many of these factors become inconsequential. However, factors including tobacco use, body size, nutritional status, disease states and current drug use may still need to be controlled to reduce variability in drug absorption and/or disposition within a given subject on different occasions. Even factors such as genetic polymorphism in drug metabolism, gender and age, normally perceived to be relevant when conducting between-subject comparison, may play a role in increasing intra-subject variability. While the reasons for controlling some of these factors are quite apparent, the others may not be so easily justified as one of the criticisms of the manner in which BE studies are conducted is that the subject population being tested does not represent the general population adequately. However, the already high degree of variability observed with some drugs and/or possible safety concern argue against decreasing control in non-formulation related variables in the conduct of BE studies.

Variability in pharmacokinetic parameters: A regression model to predict intrasubject variability for bioequivalence studies.

E. Masson, Gino Roy, Claude Lapointe, Zohreh Abolfathi, François Vallée and Marc LeBel, Anapharm Inc., Sainte-Foy, Québec, Canada

Purpose: An adequate estimation of the intrasubject variability of the pharmacokinetic (PK) parameters is needed to properly estimate the sample size required for a crossover bioequivalence (BE) study. However, it is rarely available from the literature. The purpose of this study was to evaluate the performance of a linear regression model built to predict intrasubject coefficients of variation (ISCVs), based on pooled CVs and PK characteristics of the studied drugs. Methods: Data from 31 single dose crossover BE studies conducted in at least 12 subjects and involving 26 drugs were used; studies which had mean ratio (test/reference) outside 80-125% were excluded. A total of 108 PK parameters for both parent compound and metabolites (Cmax and AUCs) were derived from these studies. ISCVs were derived from ANOVA on ln-transformed parameters. Pooled CV (%) was estimated as SD/mean X 100.Results. Mean ISCVs and pooled CVs are displayed in Table 1. The final multiple linear regression model (A) included the following significant covariables (pooled CV, PK

parameter, genetic polymorphism, first-pass metabolism, hepatic/renal elimination and fast/fed study. Its adjusted r-square was 0.66. Model (B) utilized the ratio ISCV/PCV to predict Cmax, and AUC. Using Model A, 73.1% of the predicted ISCVs were within the range of ±5% of observed values, compared with 52.8% for the mean ISCV/PCV ratio model (B). Model A was found to be significantly more precise and accurate than Model B.

Table 1. Characteristics of the PK Parameters

Parameters:	Intrasubject CV (%)	Pooled CV (%)
	Mean ± SD	
All parameters	15.1 ± 9.6	37.4 ± 20.7
Cmax	19.2 ± 11.7*	33.4 ± 17.2
AUCs	13.1 ± 7.7	39.3 ± 22.1

*$p < 0.05$ *vs AUCs*

Conclusion. As expected, Cmax is more variable than AUC. In addition, the ratio of ISCVs/PCV is higher for Cmax (57%), than for AUC(33%). Although, the mean ISCVs/PCV ratio can be used to estimate the ISCV of Cmax, or AUC, the multiple regression model (A) is more precise, and accurate. Thus, this model could be used to properly estimate the ISCV, and estimate sample size required for BE studies.

Liposomes for solving difficult drug delivery problems.

T.M. Allen, Department of Pharmacology, School of Medicine, University of Alberta, Edmonton

Many drugs fail to reach the clinic or fail early in clinical trials because of problems with delivery, pharmacokinetics or toxicity. Problems can include poor aqueous solubility, vesicant properties, lack of stability during storage, rapid metabolism, unfavorable pharmacokinetics, non-specific toxicities and poor distribution to target tissues. Drug delivery systems such as liposomes can help solve a number of problems associated with drug delivery and several liposomal drugs are approved for clinical use, with many more liposomal drugs in clinical trials. Liposomes composed of naturally occurring lipids are non-toxic, non-immunogenic and biodegradable. Both hydrophobic and hydrophilic drugs can be associated with liposomes, and drugs can be protected from enzymatic or other mechanisms of degradation while inside liposomes. Association of drugs with delivery systems like liposomes can also radically change their pharmacokinetics and ameliorate their toxicities. In general, liposome entrapment of drugs decreases their clearance and volume of distribution and increases their AUC. Long-circulating liposomes have been shown to concentrate in regions of increased vascular permeability like solid tumors and sites of inflammation and their biodistribution to normal tissues is decreased relative to non-entrapped drugs. This can lead to increased efficacy and decreased toxicities for entrapped drugs compared to non-entrapped drugs and has been found to be particularly useful for increasing the selective toxicity of anticancer drugs. The presentation will provide an overview of the current state of drug delivery using liposomal drug carriers.

P-glycoprotein, a barrier for drug absorption.

M. Piquette-Miller, Faculty of Pharmacy, University of Toronto, Ontario

Drug transport mechanisms are important in the absorption, distribution and/or elimination of many therapeutic agents. The multidrug resistance gene product, P-glycoprotein (Pgp), a membrane bound transport protein, is involved in the active cellular efflux for a large variety of clinically significant and chemically unrelated drugs. Over expression of Pgp in tumor cells has been implicated as one of the leading causes of multiple drug resistance and therapeutic failure in cancer. Recently, substantial interest in this transporter has also arisen due to the fact that Pgp is normally expressed in healthy epithelial cells of the liver, kidney, blood brain barrier and intestine where it plays an important role in drug secretion and elimination. For instance, Pgp activity in the intestine is associated with exsorption and diminished oral absorption of drug substrates and thus may significantly contribute to the low and variable bioavailability that is observed with many agents. It has been proposed that inhibition of Pgp may have potential usefulness in overcoming bioavailability problems for these drugs. Furthermore, human diversity in Pgp expression may be an important determinant of individual differences in the bioavailability and distribution of Pgp substrates. Initial studies in our laboratory indicate that expression of Pgp may be under hormonal control of inflammatory mediators released in response to metabolic stresses such as infections. At this time we are investigating the molecular events involved in Pgp downregulation. In this seminar, discussion will focus on current research which examines the influence of Pgp expression on drug absorption as well as physiological mechanisms of P-gp control.

Drug delivery in pregnant women.

J.L. Brazier, Chaire Médicament Grossesse Allaitement, Faculty of Pharmacy, Université de Montréal, Quebec

A proportion of women exists who suffer during their pregnancy or chronic diseases or acute disorders. These women have to be given short term or long term therapy with special care. Physicians must choose the most appropriate drug and define the best dosage regimen in order to ensure a good thera-

peutic outcome for the mother while providing the maximum protection against potential teratogenicity, fetotoxicity or adverse effects in the neonatal period. In some cases, the foetus has to be therapeutically treated and one of the routes for a drug to reach foetal tissues is its administration to the mother.

Drug therapy during pregnancy has to take in account a double dilemma : to treat the mother without any hazard for the foetus and to treat the foetus without endangering the mother's health.

Drug delivery in pregnant women is complicated because during pregnancy a number of physiologic and biochemical functions undergo substantial changes. These changes influence drug disposition and drug concentrations in both maternal and foetal circulation and tissues.

On a pharmacokinetic point of view the maternal-placental-foetal unit presents special challenges in terms of transport, drug exchanges between mother and foetus and metabolism. Moreover the characteristics if the maternal-placental-foetal unit are continuously modified during pregnancy increasing the difficulties to correctly deliver drug to either the mother or the foetus. These changes and challenges will be presented and discussed. Transdermal preparations offer unique opportunity to improve compliance, achieve therapeutic goal and reduce toxicity, this route of drug delivery will also be discussed. At last, a focus will be given to new opportunities given by research on gene transfer and gene therapy during pregnancy.

Challenges of drug delivery and gene therapy in cystic fibrosis patients.

S. Ito, Division of Clinical Pharmacology and Toxicology, Department of Pediatrics, The Hospital For Sick Children, Toronto, Ontario

Cystic fibrosis (CF), which is characterized by a spectrum of clinical abnormalities such as pulmonary failure and pancreatic insufficiency, is a result of dysfunction of the epithelial chloride channel, called CFTR (cystic fibrosis transmembrane conductance regulator). Therapeutic approaches range from aerosolised antibiotics to more fundamental strategies such as "protein-repair therapy" and gene therapy to introduce functional CFTR to the airway epithelia. Despite significant progress, efficient delivery of therapeutic compounds including the gene itself, which is a key to the success of the treatment, remains challenging.

Regulatory aspects relating to the development of drugs in children, women and the elderly.

A. Klein, Health Canada, Health Protection Branch, Therapeutic Products Programme, Ottawa, Ontario

Diseases as described in the textbooks are based on the assumption that these diseases, conditions or abnormalities are similar in all, provided we examine a statistically representative sample of the population. Increasingly, however, it was found that some of the assumptions underlying this may not hold universally true. Over the years it has been recognized that the characteristics of a disease will be variable depending upon a number of factors such as the disease entity itself, the racial or ethnic background of the patient, the patient's genetic makeup, his/her age, his/her sex, etc.

Similarly, assumptions had been made that, once the effect of a drug had been elucidated and its mechanism(s) of action understood, the knowledge was applicable to every patient, irrespective of age or sex or any other factor. Over the years it has been also learned that, in addition to significant inter and intra subject variability of the therapeutic effect of a drug, there is a variability in the properties of the agent that will depend on whether the product is administered to a child, a man, a woman, or an elderly person. There was a time that we thought of a child as merely a "small adult." These assumptions can now be safely considered myths, as each age has its peculiar characteristics for disease presentation as well as for responses to treatment.

These peculiarities present special problems to regulators whose task it is to ensure that the therapies that reach the market are safe and effective medicines, useful for all ages and all groups of individuals. The challenges are multiple and range from the simplest one of keeping abreast of knowledge, to the most complex ones that deal with the application of pahrmacokinetic and pharmacodynamic variability, to the decision of whether and when to allow market access of a product and for which group.

The talk will review the past state, the current transitional situation and look to the most desirable situation in the future. There will also be a discussion of some confounders when analysing these clinical trials together with an attempt at defining the consequences of including a more or less wide or narrow age range of subjects/patients. There will be comments on how to the decide on the most suitable way to select endpoints. It is hoped that the comments will reinforce the need to continue consultations with the Canadian Regulatory Agency and to take into account issues proper to the Canadian setting, when designing plans for drug development and preparing files for submission.

Exploring Human Diversity in
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Poster Presentations

ETOX-101 a Novel Inhibitor of *Pseudomonas aeruginosa* Exotoxin A.

Souzan Armstrong and A. Rod Merrill. Guelph-Waterloo Center for Graduate Work in Cheoistry and Biochemistry, University of Guelph, Department of Chemistry and Biochemistry, Guelph, Ontario, Canada N1G 2W1.

Purpose: *Pseudomonas aeruginosa* is a gram negative opportunistic pathogen involved in disease-inducing infections of immuno compromised patients suffering from cystic fibrosis, cancer or burns. The bacterium produces a large number of virulence factors which contribute to its pathogenesis. One of the most potent virulence factor associated with *Pseudomonas aeruginosa* is exotoxin A (ETA). ETA belongs to class of toxins known as mono-ADP-ribosyl transferases, which exerts its effect via ADP-ribosylation of a dipthamide residue on elongation factor 2 (eEF-2). Ribosylated eEF-2 blocks the growth of nascent polypeptide chain, hence inhibiting protein synthesis leading to cell death. Antibiotic treatments in eradication of infections *Pseudomonas aeruginosa* are often ineffective, as a result the intoxication pathway of ETA has been a target for development of potent and selective inhibitors. **Methods:** The inhibition of enzymatic activity of ETA was evaluated by a fluorescence based assay using e-NAD^+ as the substrate. **Results:** Here we demonstrate the effect of a novel inhibitor of ETA. Kinetic studies show that ETOX-101 inhibits ADP-ribosyl transferase activity by a tight binding mechanism. The IC_{50} of this compound has been determined to be ~ 113nM making it the most potent inhibitor of ETA available. **Conclusions:** The high potency of this compound makes it an attractive candidate for an effective drug design strategy.

Evaluation Of Host Response To Implanted Cross-Linked High Amylose Starch (Contramid®)

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Purpose. As cross-linked high amylose starch (Contramid®) could be used parenterally, the purpose of this study was to evaluate host reaction to subcutaneous implanted Contramid® in mice over a period of 180 days. **Methods**. Pellets (3mm diameter; 25mg) were prepared by direct compression of Contramid® and hydroxypropylmethylcellulose (16%). Seventy mice were randomly allotted in 7 groups. One pellet per animal was implanted subcutaneously on the dorsal area. Animals were clinically monitored after implantation until euthanasia on days 3, 7, 14, 30, 60, 120 and 180 post-implantation. After macroscopic evaluation, implantation sites were prepared for histological sections. The slides were evaluated by grading inflammatory reaction according to cells infiltration. Results. No mortality was observed. General behaviour was not altered. Macroscopic reaction decreased from 5mm (swelled pellet) to 2mm (fibrous nodule) from day 3 to 180, respectively. Microscopic host reaction was similar in every animal. The predominating inflammatory cells were heterophils (from day 3 to 14) and macrophages (from day 7 to 180) mainly found inside and around the pellets, respectively. Fine fibrovascular septas gently divided the implant over time causing its complete fragmentation. A loose peripheral well-vascularized connective tissue developed around pellets. Specific polysaccharides staining by periodic acid-Schiff always allowed detection of Contramid® and/or by-products firstly outside the inflammatory cells and then in macrophage cytoplasm. **Conclusion**. Contramid® is well tolerated in vivo, seems to be mainly phagocytosed by macrophages and induces a slight peripheral fibrosis suggesting a possible bi-directional diffusion between the polymer and surrounding tissues. This study provides optimistic results for parenteral cross-linked high amylose starch administration as a drug delivery system.

Antibody-Catalyzed Hydrolysis ff N-Methylcarbamate Prodrugs: Potential For Antibody-Directed Abzyme Prodrug Therapy.

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Purpose: One of the potential applications of catalytic antibodies are as catalysts for the activation of prodrugs into biologically active drugs and the selective delivery of prodrugs to targeted cells (antibody-directed abzyme prodrug therapy - ADAPT). Drugs bearing free amino groups that are essential for the biological activity of the drug (such as vinblastine, melphalan and doxorubicin) are attractive targets for ADAPT since the amino groups can be readily converted into stable and bio-inactive carbamate prodrugs which could then be converted back into their original bio-active form by abzyme-catalyzed hydrolysis of the carbamate moiety. The purpose of this work is to obtain an antibody that is capable of catalyzing the hydrolysis of N-methyl carbamate-derived prodrugs. **Meth-**

ods :A phosphoramidate transition state analogue for N-methyl carbamate hydrolysis was synthesized. Monoclonal antibodies were raised against the transition state analogue using standard hybridoma techniques. 32 hybridoma clones were selected on the basis of secreting antibodies with high binding titers for the TSA-BSA conjugate. The clones were grown up intraperitoneally in mice and pure Mab were isolated in milligram quantities from ascitic fluid by affinity chromatography on protein A-Sepharose. These monoclonal antibodies were screened for their ability to hydrolyze a model N-methyl carbamate.Results Several monoclonal antibodies were found to catalyze the hydrolysis of the model carbamate. The most efficient of these antibodies, ST51, was selected for further study. ST51 was found to exhibit Michaelis-Menton kinetics and was inhibited by stoichiometric quantities of the transition state analogue. ST51 was active at basic pH's (9-10) but did not exhibit any significant activity at physiological pH. The purified Fab fragment of ST51, generated by papain digestion, exhibited the same catalytic activity as the parent Mab. ST51 was found to catalyze the hydrolysis of a number of model prodrugs at basic pH. Conclusion Abzymes capable of catalyzing the hydrolysis of carbamate prodrugs can be obtained by raising antibodies to phosphoramidate transition state analogues. However, these antibodies do not exhibit any significant activity at physiological pH. Further studies are necessary in order to obtain abzymes that are capable of hydrolyzing N-methyl carbamates-derived prodrugs at physiological pH.

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Assessing Metabolic Diversity With Metabolic Probes Labelled With 13c.

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Various in vivo methods are used to evaluate the capacity of the liver to metabolise substrates, but they don't show direct correlation with the P450 enzymes family. Stable Isotopically Labelled (SIL) molecules can be used as metabolic probes for various enzyme system and used in breath tests.

Purpose: The purpose of the study was to validate 13C caffeine and 13C aminopyrine in different groups of subjects with characteristic CYP1A2 activity to assess the metabolic diversity of the groups and use them as test to improve subjects selection in clinical trials. **Method**: The study was carried out on 5 groups of 24 healthy adults : men no smoker, men smoker, women no smoker taking no oral contraceptive steroids (OCS), women no smoker taking OCS and women smokers taking OCS. Each subject underwent a 13C caffeine breath test (CBT) and 8 days later, a 13C aminopyrine breath test (ABT). The 13C enrichment of the expired CO2 was determined by isotopic ratio mass spectrometry (IRMS). The variations of 13C enrichment (d‰) versus time as well as the area under the d‰ = f(t) curve were determined. **Results**: Results of the breath tests show large interindividual variations in accordance with that of liver CYP450 content. Nevertheless, the parameters of CBT and ABT allowed to significantly differentiate enzyme activities between (i) men smoker and no smoker, (ii) women no smokers taking or not OCS and women taking OCS smoker or not. CBT failed to differentiate CYP1A2 activity between males and females when ABT allowed to differentiate the two groups.Our results confirm that there is no correlation between the score of both CBT and ABT with the results of the classical biochemical tests (ASAT, ALAT, ГТ, proteins, bilirubin…) performed to assess liver condition for the selection of subjects entering clinical trial. **Conclusion**: These results show that 13C caffeine and 13C aminopyrine can be used as metabolic probes for non invasive breath tests to check CYP1A2 activity (CBT) and a more global liver metabolic activity (ABT). They allow to point out induction and inhibition on drug metabolising enzymes. It has been shown that CYP450s other than CYP1A2 contribute to aminopyrine metabolism and that the score of the aminopyrine breath test is correlated to the functional hepatic mass. As (i) there is no correlation between the score of the breath tests using SIL liver metabolic probes and the classical biochemical tests used for subjects selection, (ii) unlike CBT and ABT the classical biochemical tests are not indicators of liver drug metabolising enzymes, (iii) the scores of CBT and ABT can be used as index to locate metabolising liver function of a subject with regards to a population and its diversity, these non invasive breath tests could be added to the set of tests used for subjects selection in order to improve the uniformity of the selected group.

^{13}C Basal Enrichment of Expired CO_2: Definition of Prerequisites for Kinetic Breath Tests.

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Breath tests using stable isotopes are increasingly used in diagnostic studies, in drug metabolism studies as well as to assess liver drug metabolising activities. The test measures the isotopic ratio of $^{13}CO_2$ to $^{12}CO_2$ in expired gas prior to the administration of a ^{13}C labelled substrate and at fixed intervals thereafter. The fact that the natural abundance of ^{13}C is approximately 1.1% is very important when studying the increase of ^{13}C abundance, which is often less than 0.01%. Because the studied ^{13}C enrichment is so small relatively to the natural abundance of ^{13}C, it is important to minimise the variations of basal $^{13}CO_2$ in breath. A stable ^{13}C enrichment is an essential prerequisite for kinetic breath tests. **Purpose:** The aim of the research was to study the nychtemeral variation of the ^{13}C enrichment of expired CO_2 and to define prerequisites for kinetic breath tests in order to ensure a stable ^{13}C enrichment for at least 3 hours. **Methods:** We developed a three-part protocol including: Part I: a study of the nychtemeral variation of ^{13}C enrichment in expired CO_2; Part II: a study of the effect of certain foods and beverages on the basal ^{13}C enrichment of expired CO_2; Part III: the validation of a standardised meal which does not induce any changes of ^{13}C enrichment in expired CO_2. **Results:** The results showed that important variations (up to + 6 ä‰) of ^{13}C enrichment occurred especially after food intake and physical activity. The second part allowed to verify that food and beverages containing maize (C4 plant) or sugars from maize are responsible for an increase of ^{13}C enrichment in expired CO_2. Soft drinks containing no sugar do not induce an increase of ^{13}C abundance. On the contrary, a decrease of ^{13}C enrichment is observed due to dissolved CO_2 whose ^{13}C content is very low (- 40 ä‰). In the third part, a standardised meal composed of C3 plant food items (rice, orange juice) and milk has been validated. This meal does not induce a variation of ^{13}C enrichment in expired CO_2 and reduces the intra-individual variation of this enrichment. This test meal can be used to avoid a fast of several hours prior to a breath test. This is especially important when kinetic breath tests are prescribed to pregnant woman or young children. **Conclusion:** Breath tests using stable isotopically labelled molecules as metabolic probes are non-radioactive, non-invasive tests easy to perform for assessing metabolic activities. They can be successfully used when the basal ^{13}C enrichment of expired CO_2 remains constant for the duration of the test. Objective recommendations and prerequisites have been drawn up in terms of food intake and physical activity before undergoing a kinetic breath test.

Pharmacokinetics And Metabolism Of Diltiazem: What Is The Point?

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Purpose: To determine the significance of pharmacokinetics and metabolism in the clinical use of diltiazem Methods Pharmacokinetics and metabolism of diltiazem was determined in different animal models, healthy volunteers and in patients with effort induced angina. The effects of concomitant drugs, gender, and metabolites were evaluated using standard pharmacokinetic procedures. Hemodynamic and neurohormone effects of diltiazem and its metabolites was investigated using both in vitro and in vivo techniques. **Results**: There were no gender differences in plasma concentrations although they tended to be higher in patients than in healthy individuals. There were noticeable species differences in the pharmacokinetics and hemodynmic effects of diltiazem. In rabbits, the hemodynamic effects of some of the metabolites such as M1 or M2 were smaller than diltiazem which attributed mainly to their larger clearances. Diltiazem and many of its metabolites inhibited cellular uptake ! of adenosine in vitro. In normal therapeutic doses, diltiazem also alters plasma concentrations of oxypurines probably by inhibiting cellular reuptake and oxidative metabolism of adenosine. It also attenuated the exercise induced neurohormone effects in an animal model. **Conclusion**: Despite close to two decades of clinical use, the neurohormone effects of diltiazem are only recently being realized and more studies are needed to further understand the significance of this effect in cardiovascular diseases [supported mainly by the MRC and Hoechst Marion Roussel Canada].

Development And Scale-Up Of Compression-Coated Levalbuterol Controlled Release Tablets Using Contramid® Technology.

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Purpose: Contramid® is a new starch based direct compression excipient used in controlled release solid dosage forms[1]. Although several controlled release formulations based on Contramid have been produced and tested successfully in the laboratory[2], production to industrial scale remained to be established. The objective of this work was to study the scale-up of a Contramid controlled release formulation of levalbuterol HCl. Levalbuterol HCl -the R enantiomer of albuterol- is currently under development by Sepracor Inc. for the treatment of asthma. Methods: Scale-up productions of compression-

coated tablets were performed on a 16 station Manesty Drycota 500, operated at 30 RPM. Wet granulations were performed in Glatt GPCG 1 or GPCG 15 granulator operating in top spraying mode. Dissolutions of tablets were tested in a USP type 3 apparatus (Vankel BioDis) and analyzed by HPLC. Results: Initial tests involved laboratory scale production of matrix, compression-coated, and triple layer prototypes. The release profiles obtained from these formulations showed that the matrix tablets gave an unacceptable burst followed by a profile far from linearity. This could be explained by the very high solubility of the drug (>400 g/L). The compression-coated and triple layer prototypes had a quasi linear release over about 12 hours. The compression-coated formulation was selected for the scale-up and robustness study. Initial scale-up for the compression-coated formulation was performed on the Drycota press by direct compression. This approach resulted in poor tablet uniformity that was later corrected using a wet granulation process. Several batches with a maximum sizes of 50,000 tablets were produced on the Drycota. The dissolution profiles showed outstanding tablet to tablet reproducibility. A robustness study was carried out testing the effect of core centering, tablet and core hardness, lubricant loading and levalbuterol loading. All of these parameters had no significant effect on the release profile. Dissolution was also performed under various conditions. Variations in the dissolution protocol did not affect the release profile. For example, extended residence time in simulated gastric fluid, change in agitation rate, or variation in enzymatic concentration at intestinal pH had no significant effect on the release profile. **Conclusion**: Levalbuterol controlled release compression-coated tablet incorporating Contramid was successfully scaled-up. Among the advantages of using Contramid in this formulation was very low intra-lot and inter-lot variability and excellent robustness to dissolution and manufacturing conditions.

References:

1- U.S. Patent 5,456,921 (1995).

2- I.S. Moussa and L.H. Cartilier, Int. J.Pharm. 149 (1997) 139-149.

This work has been presented at the 26th International Symposium on Controlled Release of Bioactive Materials (The Controlled Release Society, Inc.); June 21-23, 1999; Proceed. Int'l Symp. Control. Rel. Bioact. Mater., 26 (1999) pp 974-975.

Pharmacokinetics Of Cyclizine (CYC) After Single Dose Oral Administration To Human Volunteers.

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Purpose: To assess the pharmacokinetics of CYC after administration of single oral doses to healthy human volunteers and to investigate the dose-linearity of 3 single oral doses. **Methods**: The study was conducted in six healthy male subjects. The volunteers ranging in age between 18 and 20 years, each received a single oral dose of 50mg of CYC as the hydrochloride salt and serum samples were collected serially for 24 hours. Urine samples were collected throughout the serum sampling period. Serum and urine samples were prepared for analysis by solid-phase extraction and CYC quantitated by a validated HPLC method (LOQ 1ng/ml) using coulometric detection in the oxidative screen mode. Three of the volunteers participated in a dose linearity study and each additionally received 100mg and 150mg of CYC as the hydrochloride salt. The pharmacokinetic parameters of CYC were determined by non-compartmental techniques and dose-linearity assessed by least squares linear regression and two sample *t*-tests. **Results**: CYC and its demethylated metabolite, norcyclizine (NCYC) were isolated in both serum and urine samples following oral administration. The mean C_{max} (15.43 ± 3.83 ng/ml) and t_{max} (2.00 ± 0.82 hr) values following the 50mg dose were calculated directly from the serum-concentration time curves. The absorption half-life calculated by the method of residuals ranged between 20 and 40 minutes for the fasted and slowest absorbers respectively and mean terminal elimination half-life was 18.00 ± 3.15 hours. The absolute bioavailablity was determined to be approximately 50 percent using intravenous dose data from a previous study with the same volunteers and was found to be 0.47 ± 0.06. Renal clearance of CYC was low (0.0682 ± 0.00214 l/hr/kg). Total clearance and volume of distribution were calculated using the absolute bioavailability and were 0.869 ± 0.094 l/hr/kg and 22.90 ± 5.69 l/kg respectively. The mean C_{max} values for the 100mg and 150mg doses were 24.48 ± 2.04 ng/ml and 43.32 ± 5.86 ng/ml respectively. The AUC^{∞} values calculated by using the trapezoidal rule for the three single doses were 300.19 ± 39.35 ng/ml.hr (50mg), 618.65 ± 172.80 ng/ml.hr (100mg) and 921.83 ± 29.48 ng/ml.hr (150mg). Plots of dose versus C_{max} and AUC^{∞} were linear with R^2 values of 0.9605 and 0.9997 respectively. **Conclusions**: CYC is rapidly absorbed and widely distributed following oral administration of the drug. Renal clearance is negligible indicating that CYC is primarily excreted via non-renal pathways. The low oral bioavailability of CYC suggests that it may be susceptible to a first-pass metabolic process. In addition, despite the small population used for the dose linearity study, CYC does not show a tendency to exhibit non-linear kinetics.

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Differential Scanning Calorimetry (DSC) Screening Of Amoxycillin In Lipophilic Suppository Bases.

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Purpose: To ascertain whether differences in the *in vitro* dissolution of amoxycillin trihydrate (AMT) from freshly manufactured and aged suppositories could be related to changes in the DSC thermograms. **Methods**: Suppositories containing AMT in the semisynthetic bases Novata® BD/299, Witepsol®W35 and Suppocire®A32 were extemporaneously manufactured. In addition, physical mixtures of the drug and base in equivalent ratios were prepared for DSC screening. *In vitro* release studies were performed on suppositories immediately after manufacture and after one month storage (aged) at 25°C and 60% relative humidity, using USP Apparatus 1. Release of AMT was monitored using a validated HPLC method. **Results**: After 240 minute release studies of freshly prepared (aged) suppositories, 88% (99%) of the AMT was released from the Novata® BD, 85% (74%) from the Novata® 299, 44% (8%) from the Suppocire® A32 and 50% (32%) from the Witepsol® W35. The balance of AMT apparently not released was recovered when a mass balance analysis was performed. On DSC screening a shift in the melting endotherm was observed in all physical mixtures and suppositories. The appearance of an additional endotherm for the Suppocire® A32 and Novata® BD suppositories at 125°C and 135°C respectively was also observed in the physical mixtures. This additional endotherm may be indicative of a potential drug-excipient interaction. It is possible to correlate these results with the *in vitro* release findings in which the reduction in release from the Witepsol® W35 and improved release from the Novata® BD bases showed interactions, which were both unfavorable and favorable in terms of drug release. **Conclusions**: These DSC results suggest the possibility of a transformation or interaction between AMT and the semisynthetic suppository bases Novata® BD and Witepsol® W35. Although it is uncertain as to whether such a transformation might be a result of the DSC conditions, comparison of the freshly prepared and aged suppositories confirmed that a change had occurred on storage. The development of a second endotherm supported the change in drug release observed in the *in vitro* release studies and may be a result of the formation of a new solid phase, which modifies the properties of this solid dosage form.

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Binding Of pH-Sensitive *N*-Isopropylacrylamide Copolymers To Liposomes And *In Vitro* Release Studies.

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Purpose: Because cells can be induced to uptake liposomes via the endocytic pathway, pH sensitive-liposomes are a promising approach for allowing rapid content release into the cytoplasm, thus avoiding premature degradation of labile compounds. Recently, it was shown that copolymers of *N*-isopropylacrylamide, methacrylic acid and octadecylacrylate grafted on conventional or sterically stabilized fluid liposomes (SSL) could trigger a rapid release of 25% of the liposomal content. The present study describes the binding characteristics, as well as, the optimized release of this novel polymeric-based pH-sensitive system. **Methods:** Liposomes were prepared by the reverse-phase evaporation method followed by repeated extrusion through 0.1 mm pore size membrane. Liposome formulations were incubated overnight at 4°C with synthesized copolymers in solution. Binding was determined by measuring the ratio of free/labeled copolymer after separation on a Sepharose 2B column. *In vitro* release studies were performed by following the increase in fluorescence of a previously quenched fluorescent dye. **Results:** Binding studies demonstrated saturation of the liposome surface at a copolymer-to-lipid ratio of 0.12. The release studies showed that the presence of copolymer triggers a significant release of the tracer between pH 5.5 and 4.9, which can be directly correlated with the phase transition pH of the copolymer. Lack of binding of the copolymer to the SSL resulted in limited release of the dye, which has been overcome by incorporating the copolymer during the liposome preparation procedure, allowing a significant increase in binding efficiency and an improved release. **Conclusion**: This novel pH-sensitive system may eventually lead to a more efficient intracytoplasmic drug delivery *in vivo*.

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The Effect Of Association Of Diclofenac Acid With Sodium and Phospholipid On The Gastrointestinal (Gi) Toxicity.

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Purpose: To evaluate whether incorporation of phospholipids reduces GI toxicity of non-steroidal anti-inflammatory (NSAID) diclofenac. **Methods**: The GI toxicity of diclofenac acid associated withdipalmitoyl phosphatidyl choline (DPPC), diclofenac Na and diclofenac acid were assessed in rats. GI permeability tests with sucrose and Cr-EDTA as permeability probes were used as surrogate marker of NSAID-induced GI toxicity in upper and lower GI tract, respectively. Solutions containing sucrose and Cr-EDTA was administered orally to male Sprague Dawley rats at 1 and 3 h post-single 10 mg/kg dose of either diclofenac acid, or diclofenac Na as well as diclofenac-DPPC complex. **Results**: Diclofenac Na induced significant increased upper GI permeability at 1 and 3h postdose. Diclofenac acid and diclofenac complex on the other hand did not induce significant increased upper GI permeability at 1h. However, at 3h these formulations significantly increased upper GI permeability. In lower GI tract, the induced increased permeability was significant at 1h poastdose for all formulations. At 3h the increased lower GI permeability was not significant for dilofenac Na, while both diclofenac acid and complex of diclofenac significantly enhanced lower GI permeability. **Conclusion**: The increased upper and lower GI permeability of diclofenac acid associated with phospholipid is similar to diclofenac acid alone. However, the pattern of increased permeability of diclofenac Na is different from diclofenac acid alone and complex. Therefore, contrary to a previous report, the association of diclofenac with DPPC did not reduce the induced toxicity of diclofenac.

Quantitative Culture And Molecular Differentiation Of *Malassezia* Species From Different Body Sites Of Individuals With Or Without Skin Conditions.

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Purpose: This study was undertaken to correlate the quantity and species composition of clinically healthy individuals vs. patients with seborrheic dermatitis, tinea versicolor, atopic dermatitis and psoriasis. Specific objectives were:

i To quantify cultures of *Malassezia* from different body sites in humans.

ii To determine whether more than one species of *Malassezia* can be isolated from the same individual identified on the basis of polymerase chain reaction-restriction endonuclease analysis (PCR-REA) system.

iii To determine whether the quantity and species composition of normal individuals differ from those of patients with various skin conditions.

Methods: Eighteen clinically healthy individuals (without any dermatoses) and 8 patients with each of the following skin conditions, seborrheic dermatitis, tinea versicolor, atopic dermatitis and psoriasis, were included in this study.

For quantitative culturing contact plates filled with a special culture media were used to obtain samples from five body sites (scalp, forehead, forearm, trunk and thigh) of all individuals in this study.

For molecular differentiation two genomic regions namely, the large subunit (LSU) of the ribosomal gene and the internal transcribed spacer (ITS) region, were amplified by PCR and further restriction analysis of the amplified regions were used for differentiating *Malassezia* species. **Results**: Positive cultures were obtained from all body sites. The number of colony forming units (CFUs) obtained from the upper body especially from the scalp and forehead was significantly greater ($P < 0.001$) than those obtained from the lower body. Among patients with skin conditions, there was a significant difference between CFUs obtained from affected vs. non-affected body sites. Two genomic regions and three restriction enzymes proved useful in differentiating 5 of the 7 *Malassezia* species by a process of elimination. More than one *Malassezia* species was identified from different body sites of the same individuals. **Conclusions**: The results of this study show that use of contact plates is an effective method for quantitative culturing of *Malassezia* yeasts from different body sites. Both physiological and molecular tests were useful in identification of different *Malassezia* species from various body sites. But for *M. restricta*, which was isolated mainly from scalp, no other *Malassezia* species were predominantly recovered from any specific body site(s).

Drug-Disease Interaction: Interferon a aa a 2a Decreases Nifedipine Reflex Tachycardia Without Altering Pharmacokinetics.

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Purpose: To determine the effects of interferon a 2a induced inflammation on the pharmacodynamics and pharmacokinetics (PK/PD) of nifedipine. **Methods**: Male Sprague Dawley rats were either untreated (n=6) or treated (n=6) with interferon a 2a (5 x 10 4 IU) sc 12 and 1 hr prior to the study. Differential white counts were measured to determine the response of the cytokine. Nifedipine was administered as a 10 mg kg $^{-1}$ po dose which indirectly induces reflex tachycardia through a homeostatic baroreceptor mechanism in response to lowered blood pressure. Nifedipine was analyzed by a validated HPLC method. Heart rates were determined by measuring the length of the R-R interval of a lead 1 electrocardiogram. **Results**: The pharmacokinetic parameters Cl/F, AUC0-last,Cmax and t1/2 life remained unchanged, while the hematological counts and pharmacodynamic parameter was affected as shown below.

	Max. % change in heart rate	Serum Nitrite	% Lymphocyte Count	% Neutrophil Count	% Segmented Neutrophil Count
Control	33.3 ± 6.7	31.9 ± 8.7	85.5 ± 7.9	10.1 ± 4.8	2.1 ± 2.1
INF-α 2a	8.8 ± 8.9***	42.4 ± 10.5	59.1 ± 8.0***	21.6 ± 8.0*	18.7 ± 4.1***

** $p \le 0.05$, ** $p \le 0.01$, *** $p \le 0.001$*

Conclusions: Nifedipine is a calcium channel blocker indicated for hypertension and chronic stable angina, which induces reflex tachycardia in response to a lowered blood pressure. Interferon a 2a, a pro-inflammatory cytokine, had no affects on the pharmacokinetics of nifedipine while significantly decreasing reflex tachycardia. These results suggest a downregulation of calcium channels.

Disease-Drug Interactions: The Effect Of Systemic Inflammation On The Pharmacokinetics And Pharmacodynamics Verapamil And Propranolol In An Hla-B27/Human ß-2-Microglobulin Transgenic Model Of Spondyloarthropathy.

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Purpose: To determine the effects of systemic inflammation resulting from HLAB27/HB2M gene expression in transgenic (TG) rats on the pharmacokinetic-pharmacodynamic (PK-PD) relationships of verapamil (VER) and propranolol (PRP). Methods: Racemic VER and PRP (25 mg/kg) was given by gavage to transgenic HLAB27/HB2M and normal Fischer 344 rats. VER (n = 4 per group) PRP (n = 3 per group). Serial blood samples were then taken over a six hour period simultaneously with a lead I ECG. Stereospecific HPLC assays were used for both drugs. Serum nitrite was also measured as an indicator of reactive nitrogen species such as nitric oxide. Results: No significant differences in AUC $_0$® ¥ , CL/F or l_z were detected for R,S-VER or R,S-PRP.

Group	%PR	%HR	Emax	EC50
Control: PRP	27.0 ± 0.5	26.2 ± 1.5	18.9 ± 17.6	9.7 ± 10.3
TG: PRP	12.0 ± 3.2	10.9 ± 0.7	9.9 ± 2.2	13.1 ± 11.7
Control: VER	50.5 ± 12.8	22.6 ± 7.2	20.1 ± 11.4	71.3 ± 39.7
TG:VER	15.8 ± 3.2	30.3 ± 9.1	18.6 ± 7.3	96.5 ± 22.7

No significant differences were observed in serum nitrite between control and transgenic groups. **Conclusions**: In this model of systemic inflammation, no changes in pharmacokinetics were observed. This differs from other models of systemic inflammation that demonstrate downregulation of CYP450 isozymes and decreased oral clearance. Despite no change in pharmacokinetics, less dromotropic and chronotropic effects are observed for both VER and PRP. This suggests a down regulation of both L-type Ca^{++} channels and ß-adrenoreceptors. The lack of difference in serum nitrite suggests that this down regulation occurs independently of nitric oxide production.

Drug-Disease Interactions: Effect Of Interferona2a On Lidocaine Pharmacokinetics And Cardiodynamics.

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Purpose: To investigate whether inflammation caused by administration of interferona2a (INFa2a) results in altered pharmacokinetics or pharmacodynamics of lidocaine as previously reported for calcium channel blocker, verapamil. **Methods**: Lidocaine and verapamil (positive control) and placebo (n=6/group) were administered as single oral bolus doses of 90 and 20 mg/kg respectively to male Sprague Dawley rats; the test groups were inoculated with INFa2a 5.0 ´ 10^5 IU. Serial blood samples were collected via the right jugular vein for 6 hours for verapamil and 3 hours lidocaine. A modified lead I ECG was used to record the PR, RR and QT intervals. Differences in ECG intervals between placebo and INFa2a groups were determined by comparing maximum percent changes from baseline values. The QT interval was corrected for changes in heart rate and is reported as QT_c. Results. No significant differences in lidocaine pharmacokinetics were found between placebo and test groups (CL/F 226±168 vs 315±135 ml/min) and amount of metabolite (monoethylglycinexylidide) formed. Similar changes in RR and QT_c intervals after lidocaine administration were observed with placebo and test groups (RR interval 16±3% vs 16±7%; QT_c interval 25±5% vs 21±5%).

As expected, significant differences between placebo and INFa2a treated groups were found with respect to R-Verapamil pharmacokinetics (CL/F 4.7±1.4 vs 1.3±0.19 L/h), and dynamics (PR interval 23±2% vs 14±2%). **Conclusions**: Pharmacokinetics and cardiodynamics of verapamil are altered by INFa2a treatment, lack of effect on lidocaine, perhaps, is due to discrete actions of INFa2a on cytochrome P450 isozymes and cardiac ion channels. The influence of INFa2a-induced inflammation on drug action and disposition is not universal.

Effect of Protein-Calorie Malnutrition (PCM) On Drug Metabolism.

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Purpose: The effect of PCM on phase I metabolism is not well characterized. The objective of this study is to determine the effect of PCM on liver metabolic protein content and CYP 2C11 activity. **Methods**: PCM was induced by feeding male Sprague-Dawley rats (200-220g) 2/3 of the maintenance ration for a period of two months. Serum albumin was measured using a dye binding method; liver microsomal protein was measured using a Bio-Rad protein assay; and CYP was measured using a carbon monoxide binding method. The activity of CYP 2C11 was measured using 2a-hydroxylation of testosterone. Testosterone and its metabolites were measured using HPLC. **Results**:

Parameters	Control (n = 5)	Malnourished (n = 5)
Body weight (g)	470 ± 6.8	182 ± 4.5*
Liver weight (g)	18.9 ± 0.5	5.9 ± 0.9*
Serum Albumin (mg/L)	40.6 ± 2.3	39.4 ± 2.1
Microsomal Protein (mg/g liver)	43.5 ± 2.5	25.8 ± 2.6*
Total P450 (nmol/g liver)	24.1 ± 7.6	14.3 ± 4.0*
2a-Hydroxylase activity (nmol/min/mg protein)	5.31 ± 0.32	2.45 ± 0.38*

Value=mean±SEM, * P<0.05.

Conclusion: We conclude that serum albumin is not a good indicator of PCM. PCM significantly decreases the microsomal protein level, the total CYP and the CYP 2C11 activity. Since CYP 2C11 in rat and CYP 3A4 in human share similar substrates for metabolism, we postulate that PCM may have a significant effect in altering drug metabolism involving the most abundant CYP isoenzyme in human.

Impact Of Study Design On Lovastatin Bioequivalence (BE) Studies.

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Purpose. Lovastatin is a cholesterol-lowering agent administered in the lactone form that must be converted to the hydroxyacid form for activity. In humans, less than 5% of the active drug reaches the general circulation due to the extensive first pass metabolism by the liver. Thus, the bioavailability of lovastatin is low and variable. The purpose of this study was to evaluate the impact of study design on lovastatin BE studies. **Methods.** The data from 5 crossover BE studies using different designs were compared: single dose (SD) fasting (n = 2), single dose fed (n = 2) and multiple dose (MD) fed (n = 1). Intra-subject coefficient of variations (Intra CV%) was derived from ANOVA on ln-transformed parameters (AUCs and Cmax). **Results.** Multiple peaks were observed when lovastatin was administered under fasting conditions, which seems to coincide with food intake post-dose. These peaks were absent in the fed MS study. The within subject variability as evaluated by Intra CV% for AUC_{0-t}, was reduced when lovastatin was administered with food and following multiple dose administration compared to fasting conditions. **Conclusions.** The pharmacokinetics of lovastatin is more variable when the drug is administered under fasting conditions. Thus, a MD fed study design, which mimic how lovastatin is used clinically might be more appropriate when assessing BE of lovastatin formulations.

Intra CV% of the AUC_{0-t} for lovastatin and OH-Lovastatin.

	SD FAST	SD FAST	SD FED	SD FED	MD FED
Lovastatin	35.02	34.71	26.92	17.42	16.03
OH-Lovastat	27.76	23.99	23.02	20.43	25.20

Bioequivalence Study Of Lovastatin 40 Mg Tablets Under Fed Steady-State Conditions.

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Purpose. To compare the rate and extent of absorption of lovastatin 40 mg tablets by LICONSA S.A. / CEPA S.L. (Liposcler®) and Merck Sharp & Dohme (Mevacor®). **Methods.** A randomized, two-way crossover multiple-dose study with a 7-day washout between periods was conducted in 36 normal, non-smoker, and healthy males. Lovastatin was administered as a 1 x 40 mg tablet after the evening meal for 5 consecutive days. Pharmacokinetics was determined using standard non-compartmental methods and statistical analyses were performed with SAS, as per FDA/EMEA guidance's. The % of fluctuation (% Fl) was calculated as 100 x $(C_{max}–C_{min})$ / C_{av}. Lovastatin (LOV) and hydroxyacid lovastatin (OH-LOV) were quantified in plasma by LC/MS/MS. Four (4) blood sam-

ples were obtained pre-dose (Day 1, 3, 4 and 5) to assess steady-state whereas eighteen (18) blood samples were taken over 24 hours (on Day 5) to assess bioequivalence. **Results.** Mean parameters, least-squares mean ratios (Liposcler® vs Mevacor®) and 90% geometric confidence intervals (C.I.) are presented for both LOV and OH-LOV. Repeated measures analysis demonstrated that both analytes reached steady-state. **Conclusions.** All least-squares mean ratio and 90% geometric C.I. were within the 80-125% limits required by FDA and EMEA. Therefore, it can be concluded that Liposcler® 40 mg is bioequivalent to Mevacor® 40 mg, under fed steady-state conditions.

LOV	Liposcler®	Mevacor®	Ratio (%)	90% C.I.
AUC_{0-t} (pg.h/mL)	31257.67	32281.57	96.6	90.7-102.9
C_{max} (pg/mL)	7363.46	8067.92	90.0	80.7-100.4
% Fl	583.98	617.45	93.0	85.0-101.8
OH-LOV	Liposcler®	Mevacor®	Ratio (%)	90% C.I.
AUC_{0-t} (pg.h/mL)	54349.80	53680.15	103.5	93.8-114.3
C_{max} (pg/mL)	7445.21	7147.57	101.4	89.8-114.5
% Fl	326.97	331.03	97.4	89.8-105.6

Comparative Bioavailability Study Of Famotidine 40 Mg Tablets Under Fasting And Fed Conditions.

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Purpose. To compare the bioavailability of a single dose of one famotidine 40 mg tablet manufactured by Apothecon versus Merck & Co. (Pepcid®), under fasting and fed conditions. **Methods.** Subjects were randomized to a 2 sequences, 2 periods (fasting), or 6 sequences, 3 periods (limited food effect; LFE) crossover study. Famotidine plasma concentrations were measured by HPLC/UV. Pharmacokinetic parameters and statistics were performed using non-compartmental method and standard methods as per FDA guidelines. **Results.** Thirty (fasting) and twenty-two (LFE) subjects completed the study. Mean pharmacokinetic parameters, least-squares mean ratios, and 90% geometric confidence intervals (C.I.) of the test (famotidine) to reference (Pepcid®) product are presented below for the fasting and LFE studies.

Fasting	**Test**	**Ref.**	**Ratio (%)**	**90% C.I.**
C_{max} (ng/mL)	116.71	120.50	96.84	89.06-105.30
AUC_{0-t} (ng.h/mL)	654.93	657.57	98.80	90.62-107.71
AUC_{0-inf} (ng.h/mL)	714.59	716.73	99.06	91.43-107.32
LFE				
(Fed Comparison)	Test	Ref.	Ratio (%)	90% C.I.
C_{max} (ng/mL)	98.78	102.62	94.95	86.82-103.83
AUC_{0-t} (ng.h/mL)	538.27	547.62	97.38	89.31-106.18
AUC_{0-inf} (ng.h/mL)	600.21	606.42	98.14	90.42-106.52

Conclusions. The 90% geometric confidence interval for the relative mean C_{max} and AUC_{0-inf} were within 80 – 125% limits as required by FDA. Therefore, under fasting and fed conditions, a single dose of one famotidine, 40 mg tablet (Apothecon, USA) is bioequivalent to a single dose of one Pepcid® , 40 mg tablet, (Merck & Company, USA). Supported by Apothecon.

Quantitation Of Etidronic Acid In Human Plasma By GC/MS.

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Purpose. Etidronate disodium is a biphosphonate administered by oral and I.V. routes used for the treatment of osteoporosis or hypercalcemia. The purpose of this study was to develop and validate a sensitive and reproducible method for the determination of etidronic acid in human plasma. **Methods.** Etidronic acid was extracted from human plasma (0.5 mL) by solid phase extraction using anion exchange cartridges. The compounds were eluted with methanol, evaporated and derivatized with BSTFA. Chromatography was achieved on a DB-1 column with helium as carrier gas. The extracts were injected into a Hewlett Packard GC/MS. The ion monitored was m/z 575. The run time was 6 minutes per sample and the analyte was quantitated by peak area using weighted linear regression (1/C). **Results.** Linearity was observed over the range of 9.99 to 2000 ng/mL (r^2 3 0.996). Between- and within-run accuracy and precision were determined on QC samples. The between-run accuracy ranged from 95.84 to 98.84% with precision (CV%) ranging from 2.66 to 3.94%. The within-run accuracy ranged from 96.43 to 99.63% with precision ranging from 1.02 to 2.06%. The specificity was demonstrated using 8 sources of drug free plasma samples. The drug was stable in plasma for 11 hours at room temperature, for 72 hours following sample processing and after 5 freeze-thaw cycles at -20°C. **Conclusions.** This method shows good linearity, precision, and accuracy for the determination of etidronic acid in human plasma. The method is sensitive and rugged, with no observable matrix interferences.

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