The Delaware Valley
DRUG METABOLISM DISCUSSION GROUP

On
Thursday June 7, 2012
At the
Sheraton Bucks County, Langhorne PA

Presents

The 2012 Rozman Symposium on:

I) Drug Metabolism Hot Topics

Non-CYP metabolism
Metabolite identification of non-radiolabeled substrates

II) Biologics

AGENDA

Morning session	**Drug Metabolism**
8:30 – 9:00 am	**Registration**
Session I	**Hot topics: Non-CYP metabolism**
9:00 – 9:40 am	**The Role of Aldehyde Oxidase in Drug Metabolism** Jeffrey P. Jones Professor of Chemistry Donald and Marianna Matteson Professor of Chemistry Washington State University
9:40 – 10:20 am	**UGT2B17 Genetic Polymorphisms Dramatically Affect the Pharmacokinetics of MK-7246 in Healthy Subjects in a First-in-Human Study** Ying-Hong Wang Research Fellow, Dept of Pharmacokinetics, Pharmacodynamics and Drug Metabolism Merck
10:20 – 10:40 am	**Coffee break**
Session II	**Hot topics: Metabolite identification of non-radiolabeled substrates**

10:40 – 11:20 am	**Hot Results from Cold Compounds: Application of Alternative Analytical Methods to Yield Excretion Balance Information Without the Use of a Radiolabel** Ernest Schubert Manager, PTS DMPK,GSK
11:20 – 12.00	**Metabolite Identification With Non-Radiolabeled Compounds: the Good, the Bad, the Ugly** Upendra Argikar Research Investigator Novartis
12.00 – 2 pm	**Poster session* Titles listed below**
12:00 – 2 pm	**Lunch**
Afternoon session	**Biologics**
2:00 – 2:40 pm	**In Vitro Assessment of Biologics DDI** Larry Wienkers Executive Director Dept of PKDM Amgen
2:40 – 2:50 pm	**Coffee break**
2:50 – 3:30 pm	**A Platform PBPK Model for Biologics** Dhaval K. Shah Senior Scientist-Modeling and Simulation Pharmacokinetics, Dynamics and Metabolism Pfizer Inc, Groton Labs, MS 8220-4573,
3:30 – 4:10 pm	**Challenges and Opportunities in Therapeutic Protein-Drug Interaction (TP-DI) Assessments - A Perspective from IQ/DMLG - TP-DI Working Group** Honghui Zhou Senior Director Head of Pharmacokinetics & Pharmacometrics Biologics Clinical Pharmacology Janssen Research and Development Johnson and Johnson

***Posters**

1) **Cocktail Approach for Screening Relatively Selective UGT Inhibitors from the Chemical SampleBank.**
Arti Mathur*, Chuan Bai, Donald Tweedie, and Yongmei Li
Drug Metabolism & Pharmacokinetics, Boehringer Ingelheim Pharmaceuticals, Inc.

2) **Differential Selectivity of Efflux Transporter Inhibitors in Caco-2 and MDCK-MDR1 Monolayers: A Strategy to Assess the Interaction of a New Chemical Entity with P-gp, BCRP and MRP2.**
Kirsten Mease, Rucha Sane, Lalitha Podila*, and Mitchell E. Taub
Drug Metabolism & Pharmacokinetics, Boehringer Ingelheim Pharmaceuticals, Inc.

3) **The Impact of FcRn on Tissue Distribution of IgG1 in Mice**
Nancy Chen, Weirong Wang, Scott Fauty, Yulin Fang, Ping Lu, Lora Hamuro, Azher Hussain and Thomayant Prueksaritanant, Merck

4) ***In vitro* Evaluation of Regional Differences in Active Drug Transport and Metabolism in Rat and Human Intestine**
Wang, X., Guerra, F., Joseph, J., Huang, Y., Zhang, W., Bhoopathy, S. and Hidalgo, I., Absorption Systems

5) **Determination of Efflux Transporter Membrane Location using Caco-2 Knockdown Cells**
Weiskircher EA, McLaughlin A, Miezeiewksi B, Wang Q, Bhoopathy S, Nagar S, Korzekwa K, and Hidalgo, I., Absorption Systems

6) **Identification of Novel Domperidone Metabolites in Human by LC-ESI-MS/MS**
Amir Youssef[1], Murali Pathikonda[2], Upendra A. Argikar[3], Henry P. Parkman[2] and Swati V. Nagar[1]
[1]Department of Pharmaceutical Sciences, Temple University School of Pharmacy, [2]School of Medicine, Temple University, Philadelphia, PA, [3]Novartis Institutes for Biomedical Research, Inc., Cambridge, MA

7) **The Role of Aldehyde Oxidase in the Pharmacokinetics and Metabolism of Anticancer Drug Candidates**
Sarah Lawrence[1], Liangfu Chen[1], Peter Gorycki[1], Charles Davis[2], Konstantine Skordos[1]
[1]Department of Drug Metabolism and Pharmacokinetics, [2]Department of Cancer Metabolism: PreClinical Drug Discovery, GlaxoSmithKline. 709 Swedeland Road, King of Prussia, PA 19406

8) **Effect of Maternal Low Protein Diet During Pregnancy and Lactation on the Activity of Liver, Muscle, and Heart Carnitine Palmitoyltransferase-1 Activity in the Adult Offspring**

Veron Browne[1], Rani J. Qasem[1], Elizabeth Yablonski[1] , Jing Li[1], Hee Man Tang[1], Laura Pontiggia[2], Anil P. D'mello[1]
[1]Department of Pharmaceutical Sciences; and [2]Department of Mathematics, Physics, and Statistics, University of the Sciences in Philadelphia, Philadelphia, PA, 19104.

9) **Investigations into the Cytotoxicity of 3-(3,5-Dichlorophenyl)-2,4-thiazolidinedione (DCPT) and its Metabolites in Human HepG2 Cells**
Sadaff Ejaz, Ruy Tchao and Peter J. Harvison, University of the Sciences in Philadelphia, Department of Pharmaceutical Sciences.

Presentation abstracts

The Role of Aldehyde Oxidase in Drug Metabolism

Jeffrey P. Jones

Aldehyde oxidase (AO), while not a major drug metabolizing enzyme, has become increasingly important in drug failures in clinical trials and during development. Screening for AO activity requires the use of a human enzyme source, since animals do not represent the human condition. Screening should be done at multiple concentrations since most substrates show substrate inhibition at high concentrations. Furthermore, a number of drugs and natural products inhibit AO with almost all the drugs studied thus far showing complex inhibition patterns. The good news (GN) is that most AO substrates are also metabolized by other drug metabolizing enzymes. The bad news (BN) is that the enzyme produces reactive oxygen species, and in the presence of nitrite AO can produce large amounts of nitric oxide. Initial studies on known SNPs have shown only modest variability with a number of substrates (GN), although a rare (?) human phenotype exists that has no aldehyde oxidase activity (BN). Human liver cytosol and hepatocytes show variability in activity from one batch to the next (BN) for reasons that are not clear but appear to result from enzyme lability and poor cofactor incorporation. However, the GN is that computational models appear to work rather well at predicting both the rates and regioselectivity of AO metabolism. I will present new data on the inhibition of AO, the possible range of activity in human livers, and show correlations between computational models for intrinsic clearance and actual measured values.

UGT2B17 Genetic Polymorphisms Dramatically Affect the Pharmacokinetics of MK-7246 in Healthy Subjects in a First-in-Man study

Y. Wang,[1] M. Trucksis,[1] J. McElwee,[1] P. Wong,[1] C. Maciolek,[1] C. Thompson,[1] T. Prueksaritanont,[1] C. Gibson,[1] G. Garrett,[1] R. Delercq,[1] E. Vets,[2] K. Willson,[1] R. Smith,[1] J. Klappenbach,[1] G. Opiteck,[1] J. Tsou,[1] T. Laethem,[1] P. Panorchan,[1] L. Maganti,[1] M. Iwamoto,[1] R. Rippley,[1] P. Shaw,[1] J. Wagner,[1] J. Harrelson[1]; [1]Merck & Co., Inc., Whitehouse Station, NJ, [2]SGS Life Science Services, Antwerp, Belgium

BACKGROUND: MK-7246 is an antagonist of chemoattractant receptor on Th2 (CRTH2) cells developed for the treatment of respiratory diseases. The objective of this study was to investigate whether genetic polymorphism could contribute to the marked inter-individual pharmacokinetic variability in MK-7246 and its glucuronide metabolite (M3) observed in the MK-7246 first-in-man study.
METHODS: *In vitro* UGT studies were conducted to identify the UGT isoforms responsible for MK-7246 glucuronidation. Subjects who participated in the first-in-man study were genotyped. Dose-normalized area under the plasma concentration–time curve (AUC) and peak plasma concentration (C_{max}) values were determined, and genetic association analysis was performed.
RESULTS: *In vitro* enzyme kinetic studies suggested that UGT2B17 had high affinity for MK-7246, thus was likely the major enzyme responsible for MK-7246 metabolism in both the liver and intestine. Compared with the *UGT2B17*1/*1* wild-type genotype, *UGT2B17*2/*2* individuals, who possess no UGT2B17 protein, had 25- and 80-fold

greater mean dose-normalized AUC and C_{max} of MK-7246, respectively; and a 24-fold lower M3 to MK-7246 AUC ratio. The apparent half-life of MK-7246 was less variable between these two genotypes. Pharmacokinetics of MK-7246 was similar among subjects with at least one *UGT2B17*1* allele.
CONCLUSION: The genetic analysis confirms that UGT2B17 enzyme is the major UGT enzyme responsible for the glucuronidation of MK-7246. *UGT2B17* genetic polymorphisms are associated with highly variable pharmacokinetics of MK-7246 observed in the FIM study. The substantial effects of UGT2B17 polymorphism suggest that the potential effect of a potent inhibitor of UGT2B17 on a concomitant specific UGT2B17 substrate can not be ignored. UGT phenotyping should be performed when a compound is primarily eliminated by glucuronidation to dial out potentially serious drug interactions or large variability in pharmacokinetics.

Hot Results from Cold Compounds: Application of Alternative Analytical Methods to Yield Excretion Balance Information without the Use of a Radiolabel
William M. Hardesty, Igor Goljer, Janine M. Rogers, Caroline Sychterz, Ernest M. Schubert, GlaxoSmithKline, King of Prussia PA

Pre-clinical mass balance studies using radiolabel have been standard in the drug development process. However, given the high attrition rates of drug candidates, the choice to delay the radiolabel synthesis until later in drug development process represents a viable cost savings. This talk will describe how the ultraviolet (UV) chromophore and trifluoro methyl moiety of a drug candidate were both exploited to yield mass balance and biotransformation information from a preclinical rat ADME study before commitment to radiolabel production. The benefits and drawbacks of both UV spectrophotometry and fluorine-19 nuclear magnetic resonance (^{19}F NMR) spectrometry as quantitative analytical tools will be addressed. The impact of the data on early clinical development plans for the asset will also be discussed.

Metabolite Identification studies with non-radiolabeled compounds: the good, the bad, the ugly
Upendra A. Argikar
Novartis Institutes for BioMedical Research, Inc., 250 Massachusetts Avenue, Cambridge, MA 02139

The presentation will cover newer technologies as applied to metabolite identification studies with non-radiolabeled chemical entities. Application of higher energy collisional activation cell in order to enhance precursor ion fragmentation will be exemplified. In addition fragment based analysis of comparative *in silico* and *in vitro* biotransformation data from approximately 70 new chemical entities, aimed at gaining a deeper understanding of metabolite prediction software (MetaSite®) will be discussed. Finally, interesting aspects of metabolite quantification by ESI mass spectrometry in early drug discovery will be presented in the form of a case study.

In Vitro Assessment of Biologics DDI
Larry Wienkers

The cytokine-mediated suppression of hepatic drug metabolizing enzymes by inflammatory disease and the relief of suppression ("de-suppression") by successful disease treatment has become an important feature in the development of drug interaction labels for new biological products. In the past year, it has been proposed that in vitro information for cytokine-modulating biologics could be generated to help context and perhaps predict the likelihood of these types of Drug-Drug Interactions occurring in the clinic. In this presentation, P450 suppression and de-suppression studies on IL-6 in primary hepatocyte culture and studies using the murine collagen antibody-induced arthritis (CAIA) model will be used to probe the magnitude of P450 changes incurred by treating inflammatory disease. Preclinical P450 suppression and desuppression data will be compared to available preclinical & clinical literature to elucidate the pros and cons of preclinical experimentation on this drug-disease interaction.

A Platform PBPK Model for Biologics
Dhaval K. Shah
The presentation will focus on the development of a physiologically based pharmacokinetic (PBPK) model capable of characterizing the plasma and tissue pharmacokinetics (PK) of nonspecific or antigen specific monoclonal antibodies (mAbs) in wild type, FcRn knockout, tumor bearing and non tumor bearing mice. Scale up potential of the model will be evaluated by characterizing the mouse, rat, monkey and human plasma PK of mAbs, simultaneously. The model is able to characterize the plasma PK of mAb in mouse, rat, monkey and human with a common set of estimated parameters, whose values were close to some of the literature reported values. Apart from mAbs the model may also be capable of characterizing other macromolecular modalities without any major structural changes. Thus, a platform PBPK model has been developed that can characterize the data from many previously published mAb PBPK models simultaneously, and it provides a better alternative to over-parameterized two-pore models and an augmentation to previously published single-pore models.

Challenges and Opportunities in Therapeutic Protein-Drug Interaction (TP-DI) Assessments - A Perspective from IQ/DMLG - TP-DI Working Group
Honghui Zhou, PhD, Pharmacokinetics & Pharmacometrics, Biologics Clinical Pharmacology, Johnson & Johnson

Therapeutic proteins (such as antibody-based therapeutic proteins) have become increasingly more important in pharmacotherapy, and some of the therapeutic proteins have stood out as therapies of choice for certain immune-mediated inflammatory diseases and malignancies. Therapeutic proteins are commonly used concomitantly with other drugs and a certain amount of clinically relevant information has begun to emerge on drug-drug interaction potential of those therapies over the last several years. Nevertheless, to investigate drug-drug interaction potential for a therapeutic protein is inherently complicated and challenging. Some major gaps and challenges in assessing therapeutic protein-drug interactions (TP-DIs) have been identified by a 2010 BIO TP-DI

Survey, AAPS TP-DI Workshop in 2010, and IQ/DMLG – TP-DI Working Group. This presentation is intended to give a high-level summary of some ongoing activities in TP-DI within IQ/DMLG – TP-DI Working Group as well as highlight the most recent 'Workshop On Recent Advances In the Investigation of Therapeutic Protein Drug-Drug Interactions: Preclinical and Clinical Approaches' held on June 4 and 5, 2012.

Poster abstracts

1) Cocktail Approach for Screening Selective UGT Inhibitors from a Chemical Sample Bank

Arti Mathur, Chuan Bai, Donald J. Tweedie, Yongmei Li
Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT

One of the challenges to perform UGT phenotyping is the lack of selective inhibitors of different UGT isoforms. By now, only a few selective inhibitors have been identified and there is a need to discover more selective UGT inhibitors. An automated screening was performed for 1000 compounds to identify potential selective inhibitors of UGT enzymes. In order to screen a large number of compounds in a high throughput manner and to accommodate a very limited volume (3.6 μL) of each compound, a novel UGT probe cocktail approach was developed to measure enzyme activities of five major hepatic UGT isoforms (UGT1A1, 1A3, 1A6, 1A9, and 2B15) in one assay. In this cocktail assay, five specific UGT probes were incubated with human liver microsomes as a combination. The effect of one probe on the activities of other UGT isoforms was evaluated. In addition, a unique LC/MS/MS method was developed to quantitate metabolites of all 5 UGT probes which significantly enhanced bioanalytical throughput. This cocktail combination was used for the subsequent inhibitor screening assays. As a result, 20 compounds were identified as promising selective inhibitors of the studied UGT isoforms.

3) The Impact of FcRn on Tissue Distribution of IgG1 in Mice

Nancy Chen, Weirong Wang, Scott Fauty, Yulin Fang, Ping Lu, Lora Hamuro, Azher Hussain and Thomayant Prueksaritanant

Purpose: FcRn plays a pivotal role in IgG homeostasis, where it salvages the antibody from lysosomal degradation, preventing it from rapid systemic elimination. In addition to its protective role in antibody clearance, a few studies had suggested that FcRn may also play a role in tissue biodistribution of IgG. In this report, we investigated the impact of FcRn on tissue distribution of human IgG1 in this KO model and wild type mice.
Methods: An ^{125}I- labeled human IgG1 antibody was injected in both C57/BL6 and FcRn KO mice. The radioactivity of the antibody in the plasma and tissues was measured by a gamma counter. The concentration of the antibody in plasma was also analyzed by ELISA. The integrity of the antibody in the liver was further determined by Western Blot.
Results: Plasma area under the curve (AUC) was significantly lower in the KO mice, which is consistent with the primary function of FcRn. Furthermore, FcRn appeared to

have an increase in tissue to blood ratio in liver and spleen in a time-dependent manner in the KO mice. In the bone and kidney, only at 72 and 96h there were significant differences from the wild type animals. Western blot of liver lysate from the KO mice suggested that the antibody is likely still intact throughout the studied time course. Conclusions: These results suggest that in addition to its role in IgG elimination, FcRn could also play a role in antibody biodistribution potentially through transcytosis across the vasculature endothelial cells for some organs.

4) *In vitro* Evaluation of Regional Differences in Active Drug Transport and Metabolism in Rat and Human Intestine

Wang, X., Guerra, F., Joseph, J., Huang, Y., Zhang, W., Bhoopathy, S. and Hidalgo, I., Absorption Systems

The extent of intestinal absorption of many drugs are affected by active drug transporters such as MDR1 P-glycoprotein (P-gp), proton-coupled oligopeptide transporter (PepT1), and intestinal metabolic enzyme cytochrome P450 3A (CYP3A). The purpose of the present study is to characterize and compare regional differences in the expression and function of the membrane drug transporters P-gp, PepT1 and metabolic enzyme CYP3A in isolated rat and human intestinal tissue to better mimic the *in vivo* environment. Freshly excised duodenum, jejunum, ileum and colon from rats or human donors were muscle-stripped and mounted on Ussing chambers. Bi-directional digoxin (10 μM) permeability assays with and without the P-gp inhibitor cyclosporin A (CsA) were performed to determine P-gp functionality. The expression of P-gp, PepT1 and CYP3A mRNA was analyzed using qPCR to evaluate the concordance between expression and function. The P-gp function in rat intestine is the highest in colon and ileum, followed by jejunum and the lowest in duodenum. The presence of CsA induced a nearly complete inhibition of digoxin efflux. A similar trend was observed in human intestinal epithelia, with lower ERs than these in rat intestine. The mRNA expression of PepT1 decreased in a distal direction, with the highest expression in human duodenum samples. The highest level of expression of human CYP3A was found in jejunum, followed, in a decreasing order, by duodenum, ileum and colon. In conclusion, the *in vitro* Ussing chamber method can be used to study the expression and function correlations for intestinal P-gp, PepT1 and CYP3A. The extent of affected drug transport in isolated rat or human intestinal tissues could help predict the role of these processes in intestinal drug absorption.

5) Determination of Efflux Transporter Membrane Location using Caco-2 Knockdown Cells

Weiskircher EA, McLaughlin A, Miezeiewksi B, Wang Q, Bhoopathy S, Nagar S*, Korzekwa K*, and Hidalgo, I., Absorption Systems and *Temple University

Transport of many drugs and endogenous compounds across cell membranes is known to be controlled by efflux transporter proteins. Since efflux transporters play an important role in maintaining homeostasis and protecting the body from xenobiotic compounds, it has been suggested that these transporters may work in concert to elicit the transport of compounds out of the cell. Based on their specific location within the membrane these

transporters can act in parallel or sequentially: if both transporters are located either on the cytoplasmic membrane or inside the hydrocarbon domain of the lipid bilayer, they are expected to act in parallel and, but if one is located on the cytoplasmic membrane while the other is on the hydrocarbon domain, they are expect to act sequentially. In parallel transport, the transporters are both acting to efflux the compounds; if either transporter level is reduced, the sister transporter can act to decrease the resulting adverse effects. As an example, it has been demonstrated, in vivo, that knockout of both efflux transporters, P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP), at the blood brain barrier resulted in a synergistic, not additive, effect on the disposition of the compound[1]. In the case of sequential efflux, a compound may be taken up from the cytosol by a transporter such as Multidrug Resistance-associated Protein 2 (MRP2) and delivered to the hydrocarbon domain (intramembrane space), where it is now able to interact with a transporter located there, resulting in the efflux of the compound from the cell. In this study, we aimed to determine the membrane location of three efflux transporter proteins: P-gp, BCRP, and MRP2. Through the use of monolayers of Caco-2 cells and Caco-2 cells in which P-gp, BCRP or MRP2 were knocked down, we assessed the effect of transporter suppression of the permeability of compounds known to be P-gp/BCRP or BCRP/MRP2 substrates. In this study we have evaluated the hypothesis that the extent to which the efflux of a compound, known to be substrate of two transporters, is decreased when one of the transporters is knocked down suggests the location of the transporters in relation to each other. Additionally, we suggest that, for compounds that are substrates of a pair of efflux transporters, the affinity and/or specificity of the interaction with a transporter can dictate the ability of the cell to extrude the drug effectively in the absence of the other transporter.

6) Identification of Novel Domperidone Metabolites in Human by LC-ESI-MS/MS

Amir Youssef[1], Murali Pathikonda[2], Upendra A. Argikar[3], Henry P. Parkman[2] and Swati V. Nagar[1]
[1]Department of Pharmaceutical Sciences, Temple University School of Pharmacy, [2]School of Medicine, Temple University, Philadelphia, PA, [3]Novartis Institutes for Biomedical Research, Inc., Cambridge, MA

Purpose: Domperidone is a dopamine type 2 receptor antagonist that is used as a prokinetic agent to treat gastroparesis. After oral absorption, it is extensively metabolized in the liver. Previous studies reported oxidative metabolites of domperidone, detected by radiometric-HPLC or single quadrupole mass spectrometric (LC-MS) techniques. Our aim was to identify domperidone 'phase I' (oxidation/reduction) and 'phase II' (conjugation) metabolites with liquid chromatography (LC) and tandem mass spectrometry (MS/MS).
Methods: Gastroparesis patients (n= 11, 18-65 years old) currently being treated with domperidone at a dose of 10 or20 mg 3-4 times/day were recruited. Subjects were non-smokers and were not on other medications known to be substrates, inducers or inhibitors of cytochrome P450 (CYP) 3A4. At the start of the study, an initial urine sample was collected. Patients were then administered their usual domperidone dose. A blood sample was collected one hour after the dose. Urine was collected (entire volume collection) for 4 hours post-dose. All blood and urine samples were stored immediately upon collection

at −20°C until further analysis. *In vitro* incubations were performed in human liver microsomes (HLM) and recombinant purified CYP3A4 to characterize metabolism mediated by CYPs and UDPglucuronosyltransferases and in human liver cytosol for metabolism via sulfotransferases. Metabolite identification studies were carried out using LC- MS/MS.
Results: Seven metabolites were detected in human plasma and urine samples. Domperidone was metabolized to two mono-hydroxylated metabolites (M1,M2), a de-alkylated metabolite (M5), and a di-hydroxylated metabolite (M7). M1 was further glucuronidated and sulfated to M8 and M11 respectively. To the best of our knowledge, M7, M8, and M11 have not been reported previously. Six additional metabolites were identified in *vitro* in human subcellular fractions which comprise two additional mono-hydroxylated metabolites (M3, M4), an alcohol metabolite (M6) possibly formed from an aldehyde intermediate, and other conjugative metabolites (M9, M10 and M12).
Conclusion: In total, 12 domperidone metabolites including 6 new metabolites have been identified. Furthermore, M1 to M7 were identified in CYP3A4 incubations indicating that it contributes to domperidone metabolism. These results will ultimately allow a better understanding of domperidone disposition in-vivo in humans.

7) The Role of Aldehyde Oxidase in the Pharmacokinetics and Metabolism of Anticancer Drug Candidates

Sarah Lawrence[1], Liangfu Chen[1], Peter Gorycki[1], Charles Davis[2], Konstantine Skordos[1]
[1]Department of Drug Metabolism and Pharmacokinetics, [2]Department of Cancer Metabolism: PreClinical Drug Discovery, GlaxoSmithKline. 709 Swedeland Road, King of Prussia, PA 19406

Abstract:
Two compounds in development in the oncology therapeutic area, bearing similar structural cores, had pronounced differences in human pharmacokinetics (exposure). Investigative studies in human liver microsomes identified an NADPH independent metabolite. Studies in hepatic S9 and cytosol fractions in the absence and presence of menadione as well as metabolite structure, implicated the involvement of aldehyde oxidase (AO) in the metabolism of both compounds. However, the comparative metabolic rates revealed that the AO product's formation was approximately 30-fold higher for the compound which exhibited poor exposure in humans. We propose that AO mediated metabolism was likely responsible for the poor exposure observed in the clinic. Additional investigative studies using a methylated (blockade of AO metabolism) analog of the compound further demonstrated the involvement of AO.

8) Effects of maternal low protein diet during pregnancy and lactation on the activity of carnitine palmitoyltransferase-1 in liver, muscle, and heart of the adult offspring

Veron Browne[1], Rani J. Qasem[1], Elizabeth Yablonski[1] , Jing Li[1], Hee Man Tang[1], Laura Pontiggia[2], Anil P. D'mello[1]
[1]Department of Pharmaceutical Sciences; and [2]Department of Mathematics, Physics, and Statistics, University of the Sciences in Philadelphia, Philadelphia, PA, 19104.

Maternal low protein diet during pregnancy and lactation decreased liver triglyceride content in the adult male offspring. This decrease could be due to increased lipid utilization caused by increased oxidation of free fatty acids. The objective of the current study was to assess the status of fatty acid oxidation in the liver, heart, and muscle of 65 day old male control (C) and low protein (LP) offspring. The activity of carnitine palmitoyltransferase-1 (CPT-1), a rate limiting enzyme in fatty acid oxidation, was used to assess the status of fatty acid oxidation. The activity of CPT-1 was measured in the forward direction using a spectrophotometric method to quantitate the CPT-1 catalyzed and carnitine dependent rate of formation of acetyl coenzyme A from palmitoyl-CoA. LP offspring exhibited a higher liver CPT-1 activity [14.3±1.52 (mean $\pm$ S.D. n = 9-10) nmol/min/mg mitochondrial protein] compared to C offspring [12.3±1.83 nmol/min/mg mitochondrial protein]. There were no differences in CPT-1 activity between C and LP offspring in muscle [C: 16.2±2.1; LP: 16.4±1.3 nmol/min/mg mitochondrial protein] and heart [C: 18.9±1.9; LP: 18.2±1.9 nmol/min/mg mitochondrial protein]. Our results suggest that maternal low protein diet specifically increases hepatic fatty acid oxidation in the adult male offspring.

9) Investigations into the Cytotoxicity of 3-(3,5-Dichlorophenyl)-2,4-thiazolidinedione (DCPT) and its Metabolites in Human HepG2 Cells.

Sadaff Ejaz, Ruy Tchao and Peter J. Harvison, Department of Pharmaceutical Sciences, University of the Sciences in Philadelphia, Philadelphia, PA.

The thiazolidinedione (TZD) ring has been implicated in the hepatotoxicity of troglitazone and similar drugs used to treat type II diabetes. We previously showed that another TZD derivative, 3-(3,5-dichlorophenyl)-2,4-thiazolidinedione (DCPT), is cytotoxic in HepG2 cells. DCPT can be converted into [[(3,5-dichlorophenyl)amino]carbonyl]thioglycolic acid (DCTA), which could further degrade into 3,5-dichlorophenyl isocyanate (DPI). DCTA and DPI may contribute to DCPT toxicity. HepG2 cells were therefore treated with 200 μM DCPT, DCTA or DPI for up to 24 hr. We found that cell death was time-dependent and the earliest onset occurred with DCTA. Furthermore, DCTA was significantly more cytotoxic than DCPT or DPI, and produced a 67% loss of cell viability at 24 hr. In contrast, cell viability was reduced 47% and 27% by DCPT and DPI, respectively. Intracellular glutathione (GSH) levels were also measured for up to 8 hr. Only DCTA produced a significant decrease in GSH content. Thus, it appears that DCTA may contribute to DCPT-induced cytotoxicity in HepG2 cells. Furthermore, GSH depletion may be a factor in DCTA cytotoxicity. Additional experiments are needed to understand the mechanism of cytotoxicity of DCPT and its metabolites. Supported by PHS grant ES012499 and the USciences Pharmaceutical Sciences Department.

Speaker biographies

Jeffrey P. Jones, Ph.D.
Dr. Jones is The Donald and Marianna Matteson Professor of Chemistry at Washington State University. At present his main interests are in understanding nitrogen-iron coordination effects on binding and metabolism by cytochrome P450, and structure function and SNP characterization of human aldehyde oxidase. He was an Associate and Assistant Professor in the Departments of Pharmacology, Physiology, Biochemistry and Biophysics at the University of Rochester from 1987 through 1998 where he developed predictive models for P450 binding and metabolism. He was a postdoc in the Biochemistry Department at the University of Wisconsin from 1990-1992, where he worked on the mechanism of phosphoryl transfer in Professor W.W. Cleland's lab. He obtained his Ph.D. in the Department of Medicinal Chemistry at the University of Washington from Professor William Trager, studying the mechanism of P450 mediated oxidations. His B.S. is in Medicinal Chemistry from the University of Michigan. Dr. Jones is co-inventor, With Dr. Ken Korzekwa, of the technology licensed to Camitro Corporation for modeling human metabolism.

Ying-Hong Wang, Ph.D.
Ying-Hong Wang is a Research Fellow in the Department of Pharmacokinetics, Pharmacodynamics & Drug metabolism at Merck Research Laboratory in West Point, PA. She received her B.S. in Biochemistry from Nankai University in China, her Ph.D. in Molecular and Cellular Biology from Oregon Health Sciences University, Portland, OR. She joined Merck & Co., Inc. in 2004 after she completed her post-doc and Clinical Pharmacology Fellowship Training with Dr. Stephen Hall from Indiana University-Purdue University at Indianapolis. Her major interests are drug-drug interactions, pharmacogenetics, and pharmacokinetics/pharmacodynamics. She has authored and co-authored 14 manuscripts and 18 abstracts in these areas.

Ernest Schubert
Ernie Schubert received his M.S. in Chemistry from Emory University in 1994. He has spent his entire career as an analytical chemist in the pharmaceutical industry with an emphasis on the structure elucidation of small organic molecules using Nuclear Magnetic Resonance (NMR) spectroscopy. He has spent the last 8+ years in the Drug Metabolism and Pharmacokinetics department at GlaxoSmithKline, King of Prussia, PA, where he currently co-manages the structure identification group.

Upendra A. Argikar, Ph.D.
Upendra Argikar is a Research Investigator in the Metabolism and Pharmacokinetics Department at the Novartis Institutes for Biomedical Research, Inc. (NIBRI), in Cambridge, MA. He received a Ph.D. in medicinal chemistry in 2006 from the University of Minnesota, Twin Cities. He joined NIBRI in 2006 with a research focus on biotransformation and pharmacokinetics of new chemical entities, pre-clinical and clinical drugs. For the past several years his interests have also included conjugative metabolism by human Uridine Glucuronosyl Transferase enzymes (UGTs), a topic on which he has co-authored three book chapters. He has published several research articles and

serves as a reviewer for many scientific journals. He teaches drug metabolism at DMPK courses across NIBRI sites and serves as a guest faculty at Massachusetts Institutes of Technology (MIT), Cambridge, MA.

Larry Weinkers, Ph.D.
Larry C. Wienkers received his M.S. in Organic Chemistry from Western Washington University in 1988 and a Ph.D. in Medicinal Chemistry from University of Washington in 1993. Larry's current research interests are focused on exploring bioactivation pathways associated with small molecule drug metabolism with particular focus on the prospective application of this information to predict drug-drug interactions in the clinic. To this end, his group applies a multidisciplinary approach using organic chemistry, biochemistry and biophysical techniques to study cytochrome P450 mechanism based inhibition and the characterization of biotransformation pathways of novel therapeutics.

Dhaval Shah, Ph.D.
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