

CONCLUSION: In the first large study of continuous ICP dose-response data, we found that there is a linear relationship between mannitol dose and ICP response and that mannitol does not appreciably reduce ICP when it is not elevated.

OI-A-III

A PHARMACODYNAMIC MODEL FOR THE TIME COURSE OF TUMOR SHRINKAGE ASSOCIATED WITH GEMCITABINE CHEMOTHERAPY IN ASIAN NON-SMALL CELL LUNG CANCER PATIENTS. L. Tham, MSc, PharmD, L. Wang, MSc, R. A. Soo, MD, S. Lee, MD, H. Lee, PhD, W. Yong, MD, B. Goh, MD, N. H. Holford, MB, ChB, National University Hospital, National University of Singapore, University of Auckland, Singapore.

BACKGROUND/AIMS: This study hypothesized that a longitudinal exposure-response model that describes and predicts anticancer effect of an oncology agent on tumor growth can be established from primary lesion(s) shrinkage in non-small cell lung cancer following gemcitabine chemotherapy. This pharmacodynamic model aims to describe tumor response over time and determine if quantitative exposure metrics for gemcitabine, or that of its metabolites, dFdU or dFdCTP were better than drug doses.

METHODS: Gemcitabine was given as an intravenous infusion on days 1 and 8 every 3 weekly in combination with carboplatin, administered only on day 1 of each cycle. Carboplatin dosing was fixed at a targeted area under concentration-time-curve (AUC) of 5 min-mg/ml. Doses and areas under the concentration-time curve (AUCs) of plasma gemcitabine, dFdU and intracellular, dFdCTP in white cells, were compared to determine which best describes primary tumor shrinkage over time. Pharmacokinetic and pharmacodynamic parameters were estimated using NONMEM (version V, release 1.1).

RESULTS: Tumor response over an average follow-up period 133 days was better described by a gemcitabine dose-driven Emax, rather than a sigmoid Emax model. The pharmacodynamic parameters derived using a Gompertz model for tumor growth kinetics were 6.61 cm for baseline tumor size ($Size_0$), 1670 h for tumor turnover half-life, 8547.5 mg for gemcitabine dose ($Dose_{50}$) at 50% tumor shrinkage, 450 h for effect transit half-life, and 950 h for tumor shrinkage factor (a hypothetical effect mediator) half-life. Between subject variability for $Size_0$ and $Dose_{50}$ were 75% and 112% respectively. Mean dose administered for this study was 15% of $Dose_{50}$.

CONCLUSION: The results of this study did not show that intracellular dFdCTP concentrations in white cells were good surrogates for dFdCTP concentrations or anti-tumor activity, in tumor cells. However, tumor shrinkage of the primary lesion(s) was successfully quantified using gemcitabine doses. This shows that exposure-response models in solid tumors can add valuable information to the decision-making process in drug development by utilizing tumor size measurements conducted during early phase clinical trials to quantify and predict anti-tumor effects.

OI-A-IV

POPULATION PHARMACOKINETIC AND PHARMACODYNAMIC MODELING OF MYDRIASIS AFTER ADMINISTRATION OF ATOMOXETINE, DULOXETINE, AND REBOXETINE AS A POTENTIAL BIOMARKER FOR NOREPINEPHRINE REUPTAKE INHIBITOR. W. Byon, MS, D. Beidler, R. Duan, D. Roman, S. Chapel, M. Huttmacher, K. G. Kowalski, P. Lockwood, University of Minnesota, Pfizer Global Research and Development, Minneapolis, MN.

BACKGROUND/AIMS: Norepinephrine (NE) and serotonin have been implicated in fibromyalgia pain via their dysfunction at the descending inhibitory pain pathway (DIPP) in the brain and spinal cord. PKPD data was collected and analyzed from a study designed to determine the potential for mydriasis as a type 1 biomarker for new therapeutic agents that target the NE component of this DIPP mechanism.

METHODS: The mydriatic effect of atomoxetine (40 mg) [A], duloxetine (80 mg) [D], S,S-reboxetine (6 mg) [R], and placebo was determined in a single-dose randomized crossover study in 16 subjects with a 7-day washout period separating treatments. Plasma samples and pupil diameter were measured over 48 hours for each treatment. PKPD modeling was performed using NONMEM to characterize the time course of mydriasis. Posterior predictive checks evaluated the compatibility of the data and model. The potency relative to duloxetine was determined based on EC50 estimates and protein binding data and compared with in vitro binding studies.

RESULTS: All drugs were described by a one-compartment model with first order absorption and elimination. An effect compartment model characterized the delay between plasma concentrations and change in pupil diameter. The model included two fixed effects for the baseline (E_0) score to reflect the study design. The change in the pupil diameter relative to effect site concentration was characterized by an Emax model with one Emax (95% CI) of 1.74 mm (1.22–2.26) for all drugs. The equilibration rate constants (95% CI, equilibration half-time) were 0.031 hr^{-1} (0.005–0.062, 22 hr) for A, 0.657 hr^{-1} (0.081–1.233, 1.1 hr) for D, and 0.089 hr^{-1} (0.039–0.138, 7.8 hr) for R. The relative potencies from the in vivo and in vitro methods are as follows:

| Clinical Data | NE Receptor Binding Assay Data | | | | |
|---------------|--|---------------------------------|-----------------------|---------|-----|
| | EC50 _{total} (95% CI) (ng/ml) | EC50 _{unbound} (ng/ml) | Relative Potency (RP) | Ki (nm) | RP |
| Drug | | | | | |
| A | 77.6 (0–199) | 1.0 | 3.2 | 1.8 | 9.4 |
| R | 16.7 (3.55–29.9) | 0.3 | 10.2 | 2 | 8.5 |
| D | 65.1 (15.7–115) | 3.3 | 1.0 | 17 | 1.0 |

CONCLUSIONS: The study showed that mydriasis can be used as a type 1 biomarker for compounds with NE reuptake inhibition. Relative potency estimates from the study were consistent with estimates from preclinical binding assays except for atomoxetine which had poor precision. Based on the equilibration half-time and relative potency estimates, duloxetine was the fastest acting drug and reboxetine was the most potent drug.

OI-B-I

GENETIC POLYMORPHISMS IN HUMAN ORGANIC CATION TRANSPORTER 1 (OCT1) ARE DETERMINANTS OF METFORMIN DISPOSITION AND RESPONSE. Y. Shu, MD, C. Brown, PharmD, R. P. Owen, PhD, S. Zhang, PhD, R. A. Castro, MD, E. T. Lin, PhD, J. Lo, MD, E. G. Burchard, MD, C. M. Brett, MD, K. M. Giacomini, PhD, University of California at San Francisco, San Francisco, CA.

BACKGROUND/AIMS: We and others previously demonstrated that human *SLC22A1* gene, which encodes organic cation transporter 1 (OCT1), is highly polymorphic in human populations. Metformin is a widely used anti-diabetic agent, and has been characterized as an OCT1 substrate. The goal of this study was to determine whether the genetic variants of OCT1 alter the disposition and response to metformin in cells and in humans.

METHODS: Stable HEK-293 cells expressing empty vector, OCT1-reference, and twelve OCT1 nonsynonymous variants were generated respectively. Metformin uptake was measured, and immunoblots were performed to examine the activation of AMPK and ACC by metformin in the stable cells. After informed consent was obtained, twenty healthy volunteers with different OCT1 genotypes were recruited into an open-label clinical study conducted in the General Clinical Research Center at San Francisco General Hospital. Metformin pharmacokinetics and plasma glucose concentrations from oral glucose tolerance tests (OGTT) were compared between volunteers who carried a decreased function OCT1 variant (variant volunteers,

n = 12) and those who carried OCT1 wild-type alleles (wild-type volunteers, n = 8).

RESULTS: Compared to OCT1-reference, seven OCT1 variants exhibited significantly reduced metformin uptake in cells. Correspondingly, phosphorylation of AMPK and ACC by metformin was reduced in cells expressing the reduced function variants. In the clinical study, similar plasma glucose levels for base-line OGTT were observed between the variant volunteers and the wild-type volunteers. In contrast, following metformin dosing, the variant volunteers had significantly higher plasma glucose levels for most of the sampling time points during OGTT compared to the wild-type volunteers. These differences resulted in a significantly greater glucose AUC in the variant volunteers compared to the wild-type volunteers (21400 ± 2290 vs 18300 ± 1600 min-mg/dL, *P* = 0.004). The variant volunteers had a significantly smaller apparent volume of distribution for metformin compared to the wild-type volunteers (1170 ± 311 vs 898 ± 158 L/g, *P* = 0.04).

CONCLUSION: Genetic variation in OCT1 affect metformin distribution in the body. Importantly, *OCT1* polymorphisms modulate cellular and clinical response to metformin.

OI-B-II

BRAIN CYP2D6 IS INDUCED IN MONKEYS BY IN VIVO NICOTINE AND IS HIGHER IN HUMAN SMOKERS: A ROLE IN INTERINDIVIDUAL VARIATION? S. Miksys, PhD, A. Mann, MSc, A. M. Lee, BSc, R. Palmour, PhD, D. C. Mash, PhD, R. F. Tyndale, PhD, Centre for Addiction and Mental Health, University of Toronto, McGill University, University of Miami, Centre for Addiction and Mental Health and University of Toronto, Toronto, ON, Canada.

BACKGROUND/AIMS: CYP2D6 metabolizes many centrally acting drugs such as opiates, antidepressants, antipsychotics, MAO inhibitors and recreational drugs e.g. ecstasy. CYP2D6 also contributes to metabolism of endogenous neurochemicals e.g. serotonin, dopamine and neurosteroids. There is large interindividual variability in response to centrally acting drugs which is not always accurately predicted by drug and metabolite plasma levels. In situ metabolism by brain CYPs may contribute to this variability. Hepatic CYP2D6 is constitutively expressed and is not altered by commonly used drugs. However, changes in brain CYP2D6 could affect responses to many centrally acting drugs, and levels of endogenous neurochemicals. Here we present evidence that CYP2D6 is higher in brains of human smokers and that nicotine alone can induce monkey brain CYP2D6.

METHODS: CYP2D6 levels and cell distribution were assayed by immunoblotting and immunocytochemistry in brain regions from human smokers and non-smokers, and from monkeys treated with saline or 0.3 mg/kg nicotine s.c. b.i.d. for 18 days. (Smokers consume on average about 0.5 mg/kg nicotine/day).

RESULTS: CYP2D6 was higher (1.5–7.5-fold) in several brain regions including basal ganglia, cortical regions and cerebellum of human smokers and monkeys treated with nicotine. The changes were cell specific e.g. Purkinje cells in cerebellum and pyramidal neurons in frontal cortex. There were some regional differences between human smokers and nicotine treated monkeys. There were no changes in hepatic CYP2D6 levels.

CONCLUSION: This is the first demonstration in primate brain that CYP2D6 can be induced by a commonly used drug, nicotine. This could potentially affect a large number of people, not only active and passive smokers, but also those on nicotine replacement therapy. Induction of brain but not liver CYPs could alter the brain metabolism of a large number of centrally-acting drugs e.g. antidepressants and antipsychotics and endogenous neurochemicals such as serotonin. The expression of inducible brain CYP2D6 may contribute to interindividual variation in therapeutic response, and possibly to some neurological disorders and neurodegenerative diseases.

OI-B-III

CYP2D IS INDUCED IN RAT BRAIN, BUT NOT LIVER, BY CHRONIC NICOTINE TREATMENT: ELUCIDATION OF THE OFF TIME COURSE. J. Yue, PhD, S. Miksys, PhD, R. F. Tyndale, PhD, University of Toronto, Centre for Addiction and Mental Health, University of Toronto; Centre for Addiction and Mental Health, Toronto, ON, Canada.

BACKGROUND/AIMS: CYP2D6 metabolizes many CNS active drugs (e.g. tricyclic antidepressants), toxins (e.g. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and neurochemicals (e.g. catecholamines and steroids). CYP2D6 is higher in brains of human smokers and nicotine-treated monkeys, but it is impractical to investigate the mechanism of the induction in monkeys. We have previously shown that CYP2D is present in many rat brain regions and that in vitro enzyme activity, protein and mRNA levels are correlated. We therefore investigated the time course of induction of rat brain CYP2D by nicotine.

METHODS: Rats were treated for 7 days with saline or nicotine (1 mg/kg base s.c.), and sacrificed at 30 min, 2 h, 4 h, 8 h, 12 h, 18 h and 24 h after the last injection. The increases in CYP2D levels by nicotine were assessed quantitatively by immunoblotting and in specific neural cells using immunocytochemistry.

RESULTS: There was no significant induction of CYP2D up to 4 h after the last injection. By 8 h, CYP2D was maximally induced in cerebellum (1.4 fold, *p* < 0.01), hippocampus (1.3 fold, *p* < 0.01) and frontal cortex (1.2 fold, *p* = 0.11), then returned to control values by 12 h. CYP2D levels in thalamus (1.3 fold, *p* = 0.12) and brain stem (1.3 fold, *p* = 0.2) also trended towards being increased at 8 h. Immunocytochemistry showed cell-specific induction by nicotine in rat brain such as in neurons in striatum and hippocampus. Hepatic CYP2D levels were unchanged at all times tested (0.98 fold, *p* = 0.9).

CONCLUSION: This is the first demonstration of CYP2D induction in rat brain but not liver by nicotine. CYP2D levels were maximal at 8 h and rapidly returned to baseline, suggesting a precise regulation. This model will be useful to investigate many aspects of CYP2D induction by nicotine including molecular mechanisms and behavioral consequences. This work supports the notion that humans exposed to nicotine, including current and passive smokers and those on nicotine replacement treatment, may have increased in situ CYP2D-mediated metabolism of centrally acting drugs and toxins as well as altered endogenous neurochemical metabolism owing to the higher CYP2D protein levels in brain. In addition, these individuals may be at altered risk for neurotoxin-mediated neurodegenerative diseases, such as Parkinson's disease.

OI-B-IV

ASSOCIATION OF ENZYME AND TRANSPORTER GENOTYPES WITH THE PHARMACOKINETICS OF DOCETAXEL. S. D. Baker, PharmD, PhD, A. Sparreboom, PhD, M. Zhao, PhD, G. Cusatis, MS, R. G. Ingersoll, BS, R. H. van Schaik, PhD, A. C. Wolff, MD, M. A. Carducci, MD, J. Verweij, MD, PhD, Johns Hopkins University, Erasmus University Medical Center, Baltimore, MD.

AIMS: To evaluate substrate affinity of the anticancer agent docetaxel (Taxotere) for hepatocellular-localized solute carriers and to assess the functional significance of known single-nucleotide polymorphisms (SNPs) in genes of putative importance to the pharmacokinetics of docetaxel.

METHODS: Accumulation of [³H]docetaxel was studied in vitro using *Xenopus laevis* oocytes injected with cRNA of OATP1A2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and NTCP. A cohort of 102 patients with advanced solid tumors (median age, 60 years; 43 females, 59 males) received docetaxel as an intravenous infusion (dose range, 20 to 75 mg/m²). Genomic DNA was isolated from whole blood and analyzed for *CYP3A5**3C, *ABCB1* at the 1236C>T, 2677G>T/A (Ala893Ser), and 3435C>T loci, and SNPs in the gene encoding OATP1B3 (*SLCO1B3*) at the 334T>G (Ser112Ala) and

699G>A (Met233Ile) loci. Genotyping was performed using PCR-RFLP based methods or by direct nucleotide sequencing. Pharmacokinetic parameters for docetaxel were estimated using non-compartmental analysis. The relationships between variant genotypes and pharmacokinetic parameter estimates were evaluated with a non-parametric Kruskal-Wallis test.

RESULTS: Only OATP1B3 was capable of transporting docetaxel to a significant extent ($P = 0.04$), suggesting that this solute carrier is responsible, at least in part, for hepatocellular drug uptake. The observed frequencies for the *CYP3A5*3C*, *ABCB1* 1236T, 2677T, 2677A, 3435T, *SLCO1B3* 334G and 699A alleles were 83.9%, 50.5%, 37.4%, 6.1%, 42.9%, 81.9%, and 81.5%, respectively, and all were in Hardy-Weinberg equilibrium. Significant linkage was observed between SNPs from within the same gene, as predicted previously. The observed mean clearance of docetaxel was 26.6 L/h (individual range, 9.69 to 89.8 L/h). The *CYP3A5* and *ABCB1* genotypes or haplotypes were not statistically significantly associated with the clearance of docetaxel ($P = 0.47$ and $P > 0.16$, respectively). Likewise, no statistically significant association was observed between the *SLCO1B3* variants and any of the studied pharmacokinetic parameters ($P > 0.07$).

CONCLUSION: This study indicates that the presently evaluated variant alleles in the *CYP3A5*, *ABCB1*, and *SLCO1B3* genes do not explain the substantial interindividual variability in docetaxel pharmacokinetics.

OI-C-I

ON THE ROLE OF ABCB1 AND ABCC2 IN DIAPLACENTAL TRANSPORT OF TALINOLOL USING THE DUALY PERFUSED HUMAN PLACENTA MODEL. V. Minarikova, PhD, K. May, K. Linnemann, MD, C. Fusch, MD, W. Siegmund, MD, University of Greifswald, Department of Clinical Pharmacology, University of Greifswald, Pediatric Hospital, Greifswald, Germany.

BACKGROUND: The multidrug resistance-associated protein 2 (ABCC2, MRP2) and P-glycoprotein (ABCB1, P-gp) which are expressed in placental syncytiotrophoblasts are supposed to form a functional barrier between maternal and fetal blood circulation. Therefore, we investigated the influence of the P-gp and MRP2 inhibitor verapamil, the P-gp inhibitor PSC833 and the MRP2 inhibitor probenecid on the materno-fetal transfer of talinolol a substrate of P-gp and MRP2 using the dually perfused human placenta model.

METHODS: 37 term human placentas were obtained after non-complicated vaginal or cesarean delivery and genotyped for ABCB1 and ABCC2. 19 placentas were dually perfused for 5 hours using a well standardized technique. The materno-fetal transfer of talinolol (0.8 μ M) was studied in a controlled, randomized manner without and in presence of verapamil (30 μ M, $n = 6$), PSC833 (1.9 μ M, $n = 6$) and probenecid (10 mM, $n = 6$) the. The transport of talinolol was related to permeability of antipyrine (0.4 mM) and creatinine (1.3 mM) which cross the placenta by non-ionic diffusion. Perfusion volume, glucose consumption and lactat production were measured to supervise viability of the perfused cotyledon.

RESULTS: The permeability of talinolol relative to creatinine permeability was significantly higher in presence of verapamil (0.66 ± 0.16 vs 0.53 ± 0.09 ; $p < 0.03$) and probenecid (0.68 ± 0.13 vs 0.59 ± 0.15 ; $p < 0.03$). There was no effect of PSC833 on the diaplacental transfer of talinolol (0.48 ± 0.11 vs 0.46 ± 0.09 ; $p = 0.35$). We observed a significant correlation between MDR1 mRNA expression and MDR1 genotype in exon 26 at position 3435. In placentas with the homozygous ABCB1 3435TT polymorphism, the ABCB1 mRNA expression was significantly lower than in placentas with the 3435CC wildtype (1.89 ± 1.32 vs 5.36 ± 3.46 ; $p = 0.037$) and heterozygous type 3435CT (1.89 ± 1.32 vs 8.10 ± 12.8 ; $p = 0.035$).

CONCLUSION: MRP2 in contrast to P-gp plays a significant role in diaplacental transport of the β -adrenergic blocker talinolol.

OI-C-II

RISK MANAGEMENT DURING DRUG DEVELOPMENT: PREGNANCY PREVENTION STRATEGIES EMPLOYED IN CLINICAL TRIALS SUBMITTED TO THE FDA. E. Pinnow, P. Scott, J. Derbis, T. Toigo, K. Uhl, FDA, Office of Women's Health (OWH), FDA, Office of Special Health Issues (OSHI), Rockville, MD.

BACKGROUND: The FDA requires clinical trials to include a representative sampling of the population that will likely use the drug. Although women may not be explicitly excluded, restricting inclusion to females of non-childbearing potential (non-CBP) may hinder female participation. These exclusions persist despite the contraceptive requirements that can be imposed for trial participation. The purpose of this study is to describe the contraceptive requirements for women and men participating in pharmacologic clinical trials.

METHOD: The study used an OSHI database that included information on trial phase and inclusion by gender. Eight-hundred-eighty-three new commercial drug protocols submitted to the Center for Drug Evaluation and Research, between January 1–April 30, 2002 were identified. A subset of 711 protocols for non-sex specific indications was reviewed by Office of Women's Health to abstract information on contraception requirements.

RESULTS: One-hundred-twelve (15.8%) protocols excluded females of childbearing potential (FCBP). FCBP were excluded in 24.6% of phase 1, 13.5% of phase 2, 4.7% of phase 3 and 10.3% of phase 4 trials. Of 112 protocols that excluded FCBP, 38 included only males, 18 included only females (all non-CBP), 56 included males and females of non-CBP, and 599 included both males and females. Of the 599 protocols that included both sexes, contraception for female participants was required in 554 (92%) and in 74 (12%) for male participants. Of the protocols with a contraception requirement for women, 197 contained vague language (e.g. "acceptable", "effective" or "adequate" contraception) and 357 contained a list of acceptable method(s). Of the 357 protocols, 311 required one or more approved contraception method and 46 required at least two combined methods of contraception.

CONCLUSION: Contraception for women is generally required for participation in drug trials. It is routinely imposed at all trial phases and may involve more than one method. Men of reproductive age are less likely to have similar restrictions. Inadequate participation of females of child-bearing potential is an important risk management issue. Broad patient representation in clinical trials is essential to ensure the safety and efficacy of FDA regulated products in diverse populations.

OI-C-III

EXAMINATION OF ACETAMINOPHEN PROTEIN ADDUCTS (APAP-CYS) IN CHILDREN AND ADOLESCENTS WITH ACETAMINOPHEN OVERDOSE. L. P. James, MD, P. M. Simpson, PhD, L. Letzig, BS, G. L. Kearns, PharmD, PhD, J. A. Hinson, PhD, Arkansas Children's Hospital Research Institute and University of Arkansas for Medical Sciences, Children's Mercy Hospital, University of Arkansas for Medical Sciences, Little Rock, AR.

BACKGROUND: We previously reported that measurement of acetaminophen (APAP) protein adducts by an HPLC-EC assay was a sensitive and specific measure of APAP-related acute liver failure (ALF) in children.

METHOD: Relationships between adducts (APAP-CYS) and clinical/laboratory parameters in 75 children and adolescents ($n = 75$; median age 13.3 years; range 0–17 years; 58 females) with APAP overdose were examined. Serum samples were obtained by convenience sampling during the time of routine clinical monitoring for APAP overdose and APAP-CYS was measured by HPLC-EC. The diagnosis of APAP overdose was determined by history of toxic dosing (>150 mg/kg APAP) or elevated serum APAP levels at admission. Management of the overdose was determined by the treating physician. The severity of APAP toxicity was stratified as none to