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PT-1

CRANBERRY JUICE DELAYS MIDAZOLAM ABSORPTION IN HEALTHY VOLUNTEERS. N. Ngo, PharmD, E. Dees, MD, M. Paine, PhD, University of North Carolina, School of Pharmacy, University of North Carolina, School of Medicine, Chapel Hill, NC.

BACKGROUND/AIMS: Cranberry juice (CBJ) is growing in popularity as a natural alternative for the prevention of urinary tract infections. CBJ has also been shown to inhibit the enteric CYP3A-mediated metabolism of nifedipine *in vivo* in rats, as well as in human liver microsomes. Accordingly, the effects of CBJ on the pharmacokinetics of the CYP3A probe substrate, midazolam, were evaluated in healthy volunteers.

METHODS: A randomized, cross-over, open-label study was conducted at the University of North Carolina General Clinical Research Center. CBJ was prepared by diluting CBJ concentrate (R.W.Knudsen®) with water to yield 200% (double-strength) juice. Healthy volunteers (n = 6) were pre-treated with 3 glasses (240 mL each) of CBJ or water, each separated by 15-minute intervals. Midazolam syrup (5 mg) was given with the third glass. Blood (10 mL) was drawn prior to dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, and 96 hours post-dose. Plasma midazolam concentrations were measured by HPLC/MS. Midazolam pharmacokinetics were determined by non-compartmental methods using WinNonlin®.

RESULTS:

PHARMACOKINETICS OF MIDAZOLAM IN HEALTHY VOLUNTEERS

Measure	Geometric Mean (CV %)		CBJ/Water Ratio	P-Value*
	Water	CBJ		
AUC _{0-∞} (nmol/L*hr)	266 (33)	338 (37)	1.27 [1.02–1.58]	p = 0.08
Cl/F (L/h)	58 (33)	46 (37)	0.79 [0.63–0.98]	NS
K _{el} (h ⁻¹)	0.18 (45)	0.18 (28)	1.00 [0.66–1.53]	NS
C _{max} (nmol/L)	123 (32)	60 (17)	0.49 [0.36–0.66]	p < 0.05
T _{max} (h) [median (range)]	0.5 (0.25)	3.5 (1.5–4.0)		p < 0.005**

*Paired Student's t-test.

**Wilcoxon signed-rank test.

*Abstracts appear in presenting order. The presenting author's name appears with underline. **PI**, **PII**, and **PIII** denote Poster Session I (Thursday March 22), Poster Session II (Friday, March 23) and Poster Session III (Saturday, March 24). **OI**, **OII**, and **OIII** denote Oral Sessions I (Thursday, March 22), Oral Sessions II (Friday, March 23) and Oral Sessions III (Saturday, March 24). **PT** denotes those abstracts submitted by trainees which were selected to receive Presidential Trainee Awards; these will be displayed on Wednesday, March 21 at the Showcase of Top Trainee Abstracts. Late breaking abstracts are not published in this *Supplement*.

CONCLUSIONS: CBJ significantly delayed the rate of absorption of midazolam, as exemplified by the 50% decrease in C_{max} and 7-fold increase in T_{max}. Potential mechanisms include inhibition of an uptake process in the proximal small intestine and alterations in physico-chemical processes (*e.g.*, delayed gastric emptying and increased acidity). The slight increase (30%) in AUC, representing an increase in fraction absorbed, without a change in K_{el} in this small group of subjects suggests inhibition of enteric CYP3A as another potential mechanism underlying this drug-diet interaction.

PT-2

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. Hutmacher, 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 I 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
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 I 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.

PT-3

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.

PT-4

ATORVASTATIN INHIBITS INTERLEUKIN 1-BETA-INDUCED PRODUCTION OF EPITHELIAL NEUTROPHIL-ACTIVATING PEPTIDE FROM HUMAN ENDOTHELIAL CELLS IN A DOSE-DEPENDENT FASHION. G. J. Welder, AA, N. Chegini, PhD, I. Zineh, PharmD, University of Florida College of Pharmacy, University of Florida College of Medicine, Gainesville, FL.

BACKGROUND/AIMS: Endothelial inflammation has been implicated in cardiovascular disease (CVD). A prototypical inflammatory cytokine interleukin-1beta (IL-1β) stimulates endothelial expression of epithelial neutrophil-activating peptide (ENA-78), which may be important in early inflammatory processes in CVD. HMG-CoA reductase inhibitors (statins) reduce CVD morbidity and mortality in part due to anti-inflammatory actions. We therefore investigated whether atorvastatin inhibits IL-1β-induced ENA-78 production in human umbilical endothelial cells (HUVEC).

METHODS: We cultured HUVEC (Cambrex BioScience Inc., Walkersville, MD) to 80% confluence in growth media at physiological temperature and 5% CO₂. Treatment groups included unstimulated control, 2ng/ml IL-1β alone, and IL-1β plus atorvastatin ranging from 1–50μM. Experiments were performed in duplicate. ENA-78 levels were measured using cytometric fluorescence detection (R&D Systems, Minneapolis, MN; Luminex 100IS, Luminex Corp., Austin, TX). ENA-78 concentrations were normalized to total protein. One-way ANOVA with post-hoc Tukey was performed; P<0.05 was considered significant.

RESULTS: Constitutive and IL-1β-stimulated ENA-78 concentrations were 69 ± 13 and 4075 ± 591 pg/mg, respectively (P<0.0001). Atorvastatin inhibited ENA-78 production in a dose dependent-fashion, ranging from 38% to 99% inhibition (P<0.0001) with atorvastatin 1 μM to 50 μM. In stimulated HUVEC treated with atorvastatin ≥25 μM, ENA-78 concentrations were no different from control (P=0.98).

CONCLUSIONS: Atorvastatin dose-dependently inhibits IL-1β-stimulated release of ENA-78 from HUVEC. Our finding offers novel insight into the potential anti-inflammatory role of statins. The mechanism of our finding and clinical implications should be further investigated.

PT-5

CORRECTING FOR INCOMPLETE AND VARIABLE GALLBLADDER CONTRACTION IMPROVES THE ESTIMATE OF BILIARY CLEARANCE OF DRUGS IN HUMANS. G. Ghisellini, PhD, L. S. Vasist, PharmD, B. M. Johnson, PhD, W. D. Heizer, MD, R. J. Kowalsky, PharmD, K. L. Brouwer, PharmD, PhD, School of Pharmacy, University of North Carolina at Chapel Hill, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.

BACKGROUND/AIMS: The anatomy of the human hepatobiliary tract, and the intermittent and incomplete expulsion of bile from the gallbladder, must be considered when quantifying biliary clearance (Cl_{biliary}) of drugs. We developed a clinical method that improves the recovery of biliary secretions and corrects for incomplete gallbladder contraction. A custom made oro-enteric tube and clinical protocol were used to quantitatively collect bile and determine the Cl_{biliary} of selected probes [Tc-99m mebrofenin (MEB), Tc-99m sesatambi (MIBI) and piperacillin (PIP)] that were expected to exhibit

high, intermediate and low Cl_{biliary} , respectively, in healthy volunteers.

METHODS: Three open label studies were performed at the University of North Carolina Hospitals, General Clinical Research Center. Fourteen healthy volunteers were administered IV either 2.5 mCi MEB, 2.5 mCi MIBI, or 2 g PIP. In the case of PIP, 2.5 mCi MEB was administered IV 2 h after PIP to determine gallbladder ejection fraction. Gallbladder contraction was stimulated 2 h after administration of the Tc-99m probe with cholecystokinin-8 (CCK-8, 0.02 $\mu\text{g}/\text{kg}$, 30-min infusion). Duodenal aspirates were collected using a custom made oro-enteric tube equipped with an occlusive balloon. The probes were quantified by gamma counting (MEB, MIBI) or LC-UV/MS analysis (PIP) in blood, bile and urine over a 3 h (MEB and MIBI) or 6 h time interval (PIP). Noncompartmental methods were used to calculate pharmacokinetic parameters. Gallbladder ejection fraction was determined by gamma scintigraphy and used as a correction factor in the calculation of Cl_{biliary} .

RESULTS: Gallbladder ejection fraction in response to CCK-8 was variable, ranging from 3% to 90%. The variability in Cl_{biliary} (mean \pm SD; mL/min/kg) was consistently lower upon correction of Cl_{biliary} for gallbladder ejection fraction: 12.5 ± 3.5 vs. 16.1 ± 3.2 for MEB, 3.9 ± 2.0 vs. 5.5 ± 1.2 for MIBI and 0.012 ± 0.011 vs. 0.032 ± 0.008 for PIP.

CONCLUSIONS: This clinical method is useful to determine the Cl_{biliary} of drugs in healthy humans. Correction for gallbladder ejection fraction minimizes inter-subject variability in biliary excretion of drugs by accounting for incomplete and variable gallbladder contraction.

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PT-6

THE DOSE-RESPONSE RELATIONSHIP BETWEEN MANNITOL AND INTRACRANIAL PRESSURE IN TRAUMATIC BRAIN INJURY PATIENTS. M. D. Sorani, UCSF, San Francisco, CA.

BACKGROUND/AIMS: Brain edema, an increase in water content in the brain, plays an important role in the pathophysiology of traumatic brain injury (TBI). Edema can increase intracranial pressure (ICP) and lead to ischemia and death. Brain edema is often treated with intravenous hyperosmotic agents to draw water out of tissue and decrease ICP. Mannitol, an alcohol sugar, is the osmotic diuretic most commonly used, yet no definitive dose-response relationship has been established. The specific relationship is important since high doses increase serum osmolality and have been associated with renal failure, whereas low doses may be ineffectual. Our aim was to characterize the dose-response relationship between mannitol and ICP in TBI patients.

METHODS: In a retrospective study at an urban trauma center intensive care unit, we measured ICP using a continuous physiological monitoring system in 28 consecutive TBI patients who were given at least one bolus of mannitol. Twenty patients were given a total of 85 doses of 50 g of mannitol, and 18 patients were given 50 doses of 100 g. Some patients received both doses.

RESULTS: Average ICP was 22.0 ± 10.6 mm Hg at the time mannitol was administered, fell immediately after dosing, and continued falling for approximately 30 minutes to 15.7 ± 8.1 mm Hg across all patients. After 30 minutes, ICP in the 100 g group (15.6 ± 10.9 mm Hg) was similar to that in the 50 g group (15.7 ± 6.3 mm Hg). However at 100 minutes, ICP had increased in the 50 g group to nearly its initial value but was lower in the 100 g group (18.6 ± 7.6 v. 14.2 ± 6.7 mm Hg; $p=.001$). We plotted ICP decrease versus weight-adjusted dose and found a linear relationship ($y=12.1x-5.6$, $p=.003$). The relationship indicates that each additional 0.1g/kg mannitol achieves an additional reduction of approximately 1.2 mm Hg in ICP. When we separately analyzed doses given when patient ICP was greater than or less than 20 mm Hg, we found that doses given when ICP was high resulted in 36% or 43% decreases in ICP respectively for doses of 50 g or 100 g, while doses given when ICP was low showed only 4% or 13% decreases in ICP respectively.

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.

PT-7

POPULATION PHARMACOKINETIC-PHARMACODYNAMIC (PK-PD) MODEL OF THE VASCULAR-DISRUPTING AGENT 5,6-DIMETHYLXANTHENONE-4-ACETIC ACID (DMXAA) IN CANCER PATIENTS. J. Li, PhD, M. B. Jameson, MD, B. C. Baguley, MD, R. Pili, MD, S. D. Baker, PharmD, PhD, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Waikato Hospital, University of Auckland, Baltimore, MD.

BACKGROUND: DMXAA selectively disrupts established tumor blood vessels. The degree of DMXAA-induced tumor hemorrhagic necrosis is correlated with increased plasma 5-hydroxyindole-3-acetic acid (5-HIAA) (a metabolite of serotonin). DMXAA is being evaluated in phase II trials in combination with cytotoxics. **Objectives:** To develop a population PK-PD model that describes the time courses of plasma DMXAA and 5-HIAA (as a vascular damage biomarker) and explores the dose-concentration-effect relationship.

METHODS: The plasma DMXAA and 5-HIAA concentration data were obtained from 124 patients receiving DMXAA monotherapy as 20-min intravenous infusion weekly or every three weeks at doses ranging from 6 to 4900 mg/m^2 in three phase I trials. The PK and PD data were analyzed by nonlinear mixed effect modeling with NONMEM program. Potential covariates including age, body weight, height, body surface area (BSA), sex, liver and kidney functions were screened with general additive model analysis and tested in the PK/PD model.

RESULTS: DMXAA concentration-time profiles were well described by a 3-compartment model with saturable elimination (Michaelis-Menten kinetic). BSA and sex were significant covariates on the volume of distribution of the central compartment (V1) and the maximum elimination rate (VM), respectively, accounting for 10 and 12% of interindividual variation in these parameters. Population estimates for VM, KM (concentration at which half VM is achieved), and V1 were $122 \times (1 + 0.502 \times (2 - \text{SEX}))$ ($\mu\text{M}/\text{h}$) [SEX = 1 for males, 2 for females], 103 μM , and $8.15 \times (\text{BSA}/1.8)^{0.69}$ (L), respectively. Plasma DMXAA and 5-HIAA concentrations were simultaneously fitted by the effect compartment PK-PD model with population PK parameters fixed to the previously obtained values from the final PK model. The effect of DMXAA on plasma 5-HIAA was described by the stimulatory E_{max} model, where population estimates for baseline, E_{max} , and EC_{50} were 46.2 μM , 2.75-fold increase of the baseline value, and 659 μM , respectively.

CONCLUSIONS: DMXAA plasma disposition is characterized by a saturable elimination process. BSA-guided dosing is important. 5-HIAA can be used as a biomarker to guide dose selection. DMXAA doses of 1000 to 2000 mg/m^2 , resulting in 1.5- to 1.8-fold increase of plasma 5-HIAA, are recommended for phase II/III studies.

PT-8

β_1 -ADRENOCEPTOR POLYMORPHISMS AND ETHNICITY INDEPENDENTLY AFFECT RESPONSE TO ATENOLOL. D. Kurnik, MD, G. G. Sofowora, MD, E. A. Friedman, BS, M. Muszkat, MD, H. G. Xie, MD, PhD, P. A. Harris, PhD, L. Jiang, MS, S. M. Williams, PhD, C. Li, PhD, A. J. Wood, MD, C. M. Stein, MD, Vanderbilt University Medical School, Nashville, TN

BACKGROUND/AIMS: Variability in the β_1 -adrenoceptor gene (*ADRB1*) affects blood pressure response to β -blockers. Less responsive *ADRB1* genotypes are more common in black than white subjects. Moreover, in some studies, β -blocker monotherapy for hypertension was less effective in blacks than whites. Thus, we addressed the hypothesis that ethnic differences in β -blocker response are due to *ADRB1* genetic variations.

METHODS: 159 healthy subjects (94 whites and 65 blacks, 93 female, aged 26.8±6.4 years) participated in an open-label, single-dose study set in the Clinical Research Center. Blood pressure (BP) and heart rate (HR) were determined after 30 minute supine rest and 3 hrs after oral atenolol (25 mg). *ADRB1* genotype for the Ser49Gly and Arg389Gly variants were determined by TaqMan assay, and haplotypes assigned by expectation-maximization algorithm. Associations between baseline measures, sex, ethnicity, atenolol plasma concentrations, body mass index (BMI), and *ADRB1* genotype and haplotype and the BP and HR responses to atenolol were assessed in multiple linear regression models.

RESULTS: The responsive allele (Arg389) and haplotype (Arg389-Ser49) were more common in whites than in blacks (70.5 vs. 56.7%, $P = 0.015$, and 57.8% vs. 30.7%, $P < 0.001$). Arg389Gly genotype was associated with the decrease in SBP (7.1 ± 8.8 , 4.8 ± 9.5 , and 1.5 ± 7.8 mmHg with 2, 1, and 0 copies of Arg389, respectively; $P = 0.038$). The Arg389-Ser49 haplotype was associated with a greater decrease in SBP (6.7 ± 9.6 and 1.6 ± 7.3 mmHg for its presence and absence, respectively; $P < 0.001$) and HR (8.3 ± 6.4 and 5.0 ± 6.9 bpm, $P = 0.014$). Whites had a greater decrease in HR (8.9 ± 6.5 vs. 5.4 ± 6.2 bpm, $p < 0.001$) and DBP (4.8 ± 6.6 vs. 2.5 ± 6.7 mmHg, $p = 0.036$) than blacks; these ethnic differences persisted after adjusting for *ADRB1* haplotypes and other covariates ($P = 0.004$ and $P = 0.042$ for HR and DBP, respectively).

CONCLUSIONS: Whites have greater reductions in DBP and HR after single-dose atenolol, but this is not explained by their higher frequency of those *ADRB1* genetic variants that are associated with greater β -blocker response. Ethnicity and *ADRB1* variants independently affect cardiovascular response to atenolol.

PT-9

GENE RE-SEQUENCING AND FUNCTIONAL GENOMICS OF FOLYLPOLYGLUTAMATE SYNTHASE (FPGS). T. A. Leil, PhD, A. A. Adjei, PhD, C. Endo, MD, O. E. Salavaggione, MD, G. K. Dy, MD, J. M. Reid, PhD, M. M. Ames, PhD, A. A. Adjei, MD, PhD, Mayo Clinic, Rochester, MN.

BACKGROUND: FPGS is a key enzyme in folate and anti-folate metabolism. It is present in both cytosolic and mitochondrial forms, both of which catalyze the polyglutamation of pteroyl-glutamate. This allows folate and anti-folate compounds to be retained within the cell and increases their affinity for target enzymes in the folate pathway. Genetic variation in the human FPGS gene has potential to impact anti-folate therapeutic efficacy in patients and folate utilization in the general population. We have re-sequenced the FPGS gene from 240 individual DNA samples and characterized the functional genomics of three non-synonymous coding single nucleotide polymorphisms (cSNP's).

METHODS: Re-sequencing of the FPGS gene was performed on Coriell DNA samples from 240 individuals of four different ethnic populations. Three non-synonymous cSNP's of FPGS were expressed in the cytosolic form of the protein in AuxB1 cells and numerous functional parameters were measured.

RESULTS: Of the 34 SNP's identified by gene re-sequencing, five were non-synonymous cSNP's that resulted in alteration of the FPGS protein sequence: F13^{Mit}L, V22^{Mit}I, R466^{Mit}/424^{Cyt}C, A489^{Mit}/447^{Cyt}V, S499^{Mit}/457^{Cyt}F. When expressed in AuxB1 cells, the A447V variant was similar to WT FPGS in nearly all functional parameters, while the R424C and S457F variants were reduced approximately 2-fold in protein expression. The in vitro catalytic efficiency of these two FPGS allozymes was also reduced: by 4.7 fold (R424C) and 2.8 fold (S457F) with glutamic acid as a substrate; and by 2.2 (R424C) fold and 2.3 fold (S457F) with methotrexate (MTX) as a substrate. Additionally, the in vitro enzyme velocity at saturating pemetrexed (PMX) concentrations was reduced by 1.6 fold for the R424C variant, and 2.6 fold for the S457F variant. AuxB1 cells harboring the cytosolic forms these two FPGS isoforms displayed a 4.3 fold increase in the EC50 for folic acid.

CONCLUSIONS: Here we describe the first comprehensive re-sequencing and functional genomic study conducted on the FPGS gene. We discovered five cSNP's, two of which alter the in vitro kinetics of the FPGS enzyme and affect folic acid utilization of cells expressing the allozymes in culture. Individuals carrying these polymorphisms may be at higher risk for folic acid deficiency and for toxicity during anti-folate therapy.

PT-10

THE EFFECTS OF THE NOVEL CB1 ANTAGONIST AVE1625 ON THC-INDUCED CHANGES ON CENTRAL NERVOUS SYSTEM EFFECTS AND HEART RATE. L. Zuurman, MD, MSc, C. Roy, MD PhD, R. C. Schoemaker, PhD, G. Asset, PhD, A. Amatsaleh, MD, L. Guimaraes, PharmD, J. Pinquier, MD, A. F. Cohen, Professor, MD, J. M. van Gerven, Professor, MD, Centre for Human Drug Research, Aventis-Pharma Recherche-Developpement, Aventis-Pharma Recherche-Développement, Leiden, The Netherlands.

BACKGROUND: CB1 antagonists are potentially useful in the treatment of obesity and associated risk factors, smoking cessation and cognitive impairment. AVE1625 is a new CB1 antagonist with high affinity for the CB1 receptor. The aim of this study was to determine the ability of AVE1625 to antagonize the effects of tetrahydrocannabinol (THC), the active ingredient of cannabis and a CB1 receptor agonist.

METHODS: This was a double blind, randomized, six-way, placebo-controlled, partial cross-over study in a specialized phase I-unit. Each of the 36 subjects received four out of the six available treatment combinations. On each study day a single oral dose of AVE1625 (20, 60 or 120 mg) or placebo was administered. Three hours later, four consecutive doses of THC (2, 4, 6 and 6 mg) or placebo were inhaled at one-hour intervals, using a Volcano[®] vaporizer. Subjects used cannabis less than once a week and all had a negative THC screen on each study day. The washout period was at least 14 days. Pharmacodynamic measurements (body sway, Visual Analogue Scales (VAS) and heart rate) were performed frequently on each study day. These methods were previously shown to be responsive to THC (Zuurman L et al., Br J Clin Pharmacol 2005;59:625).

RESULTS: Analysis was performed using mixed model ANOVA with baseline values as covariate. Complete or nearly complete inhibition of THC induced effects was observed after 60 and 120 mg AVE1625 on VAS 'alertness', 'feeling high' and 'external perception', on body sway and on heart rate. For VAS 'feeling high', VAS 'external perception' and heart rate, inhibition was also complete with 20 mg AVE1625. For body sway, the average percentage inhibition (compared to THC-effects alone) with 95% confidence intervals was 61% (22, 100); 73% (32, 113) and 74% (33, 114) for AVE1625 administered at 20, 60 and 120 mg respectively and for VAS 'alertness': 61%; (25, 97); 76% (37, 114); 94% (52, 136) for the doses of 20, 60 and 120 mg respectively. AVE1625 did not show any CNS or heart-rate effects by itself.

CONCLUSION: This study revealed that AVE1625 penetrates the brain and completely antagonizes THC induced effects with doses at or above 20 mg. Considering the lower affinity of endocannabinoids compared to THC, these findings suggest that a AVE1625 dose below 20 mg will suffice to antagonize the endogenous effects of endocannabinoids.

PT-11

A NOVEL GENOME-WIDE APPROACH TO IDENTIFY GENETIC VARIANT THAT CONTRIBUTE TO CHEMOTHERAPY-INDUCED CYTOTOXICITY. R. S. Huang, PhD, S. Duan, PhD, W. K. Bleibel, E. O. Kistner, PhD, T. A. Clark, T. X. Chen, A. C. Schweitzer, J. E. Blume, M. E. Dolan, PhD, University of Chicago, Affymetrix Inc., Chicago, IL.

BACKGROUND/AIMS: The polygenic nature of the sensitivity to drugs has limited the success of candidate gene approaches. We present a novel genome-wide model utilizing human lymphoblastoid

cell lines from the International HapMap consortium, of which extensive genotype information is available, to identify genetic variants that contribute to chemotherapeutic agents-induced toxicity.

METHODS: Our model was based on molecular biology, integrated genotype, gene expression and phenotype as measured by sensitivity of HapMap cell lines to chemotherapeutic drug. Cell lines derived from 30 trios of European descent (CEU) and 30 trios of African descent (YRI) were utilized. Cell growth inhibition with increasing concentrations of etoposide for 72 h was determined using alamarBlue[®] assay. Gene expression in these 180 cell lines was determined using the Affymetrix GeneChip[®] Human exon 1.0 ST Array. SNP genotypes and etoposide IC₅₀ were linked through whole genome association in cell lines from combined and independent CEU and YRI populations. A second association test was performed between SNP genotype and gene expression and linear regression was utilized to evaluate the correlation between gene expression and etoposide IC₅₀.

RESULTS: Among the 387,417 SNPs tested, 49, 122 and 51 SNPs were significantly associated with etoposide IC₅₀ in combined, CEU and YRI population, respectively ($p \leq 0.0001$). Among them, 7, 56 and 6 SNPs were also significantly associated with gene expression of 6, 21 and 40 genes in combined, CEU and YRI population (Bonferroni corrected $p < 0.05$). The expression of 3, 18 and 24 genes whose expression were associated with SNP genotypes also significantly correlated with etoposide IC₅₀ in the three tested populations ($p < 0.05$). These include genes that may play a role in cancer (AGPAT2, IL1B and WNT5B) and genes not yet known to be associated with sensitivity to etoposide.

CONCLUSIONS: Using our genome-wide approach, we identified previously unknown genetic variants that contribute to cell sensitivity to etoposide-induced cytotoxicity. These genetic variants contribute to drug-induced toxicity through their effect on gene expression. This novel, unbiased method can be used to elucidate genetic variants contributing to a wide range of cellular phenotypes induced by chemotherapeutic agents.

PT-12

GENOME-WIDE ASSOCIATION STUDY TO IDENTIFY RISK FACTORS FOR DEVELOPING THERAPY-ASSOCIATED (SECONDARY)LEUKEMIA. A. A. Shinde, MD, PhD, G. Hayes, PhD, R. A. Larson, MD, R. A. Larson, MD, N. J. Cox, PhD, K. Onel, MD, PhD, University of Chicago, Chicago, IL.

BACKGROUND/AIMS: Therapy-related acute myelogenous leukemia (t-AML) is the most serious long-term complication of cancer chemotherapy. Only a subset of patients treated for cancer develop t-AML suggesting a genetic predisposition. We conducted a genome-wide association (GWA) study to investigate genetic factors associated with t-AML risk.

METHODS: A GWA scan using Affymetrix Mapping 10K arrays was performed on germline genomic DNA from 81 Caucasian individuals with t-AML and compared to previously genotyped 150 healthy Caucasian controls. Cases were adults with clinically diagnosed and cytogenetically characterized t-AML followed at the University of Chicago. Controls were 150 previously genotyped unrelated healthy Caucasian individuals. After applying quality control and checking for departure from Hardy-Weinberg equilibrium (HWE), 6600 SNP markers were available for analysis. Association p values were computed using Fisher's exact test and the false discovery rate (FDR) was estimated by permutations. The extent of LD and differences in LD between cases and controls were computed using the CCOLDD (case control LD difference). The CCOLDD method is a novel computational method developed by our group, which contrasts differences in the extent of LD between cases and controls.

RESULTS:

SNP ID	Chromosome	p Value*	Gene
rs1394384	17q12	2.55×10^{-5}	ACCN1 (intronic)
rs719293	2p16.3	3.40×10^{-5}	NRXN1 (intronic)
rs1335546	10p12.1	1.94×10^{-4}	GAD2
rs2133508	4p15.2	1.98×10^{-4}	SLA/LP
rs1394605	5q33.3	2.27×10^{-4}	SGCD (intronic)
rs1374284	2q13	2.81×10^{-4}	IL-1 gene family
rs1199098	10q21.1	6.27×10^{-4}	IPMK
rs2255408	15q24.2	6.39×10^{-4}	ETFA
rs1351865	3p26.3	8.28×10^{-4}	CHL1

Table 1. Markers associated with t-AML susceptibility. P value = association p value by Fisher's exact test

Of the candidate genes identified by this analysis, none have previously been studied in t-AML, but several participate in cellular functions that have been directly implicated in leukemogenesis. The CCOLDD method detected differences in the extent of LD in the region in several gene loci important in carcinogenesis. Notable, these were—Fragile histidine triad gene (FHIT) that has been implicated as a tumor suppressor gene, Estrogen receptor 1 ESR1, CYP39A1 and JMJD2C.

CONCLUSION: The prior identification of individuals with a genetic susceptibility for developing t-AML would be invaluable in patient selection for chemotherapy and variants identified in this study may prove to be translationally useful biomarkers in subsequent prospective clinical studies.

PT-13

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, 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.

PT-14

SULFONYLUREA DRUGS LIMIT COMPENSATORY K_{ATP} CHANNEL OPENING IN LONG QT SYNDROME. S. Sattiraju, MD, L. V. Zingman, MD, S. Reyes, BS, A. E. Alekseev, PhD, A. Terzic, MD, PhD, Mayo Clinic, Rochester, MN.

BACKGROUND: Congenital or drug-induced long QT syndrome (LQTS) is characterized by QT interval prolongation on the electrocardiogram and the occurrence of life-threatening arrhythmias or sudden death, especially under conditions of stress. Prolongation of cardiac repolarization results in increased energy consumption to maintain cellular homeostasis. Cardiac K_{ATP} channels are membrane-based metabolic sensors that are able to adjust action potential duration (APD) under conditions of increased energy demand. Here we tested the role of normal K_{ATP} channel function in the development of LQTS, and the outcome of channel blockade by oral-hypoglycemic sulfonylurea drugs on pro-arrhythmic action potential prolongation.

METHODS: Retrogradely perfused hearts were paced in the physiological range. APD was evaluated using monophasic action potential (MAP) recording. The contribution of K_{ATP} channel-dependent APD modulation was validated in wild-type (WT) and K_{ATP} channel-deficient (Kir6.2-KO) transgenic models at different heart rates, in the presence and absence of the sulfonylurea glyburide. LQTS was produced by non-specific potassium channel blockade with 4-aminopyridine (4-AP) or blockade of sodium channel inactivation with anthopleurin-A. *In vivo* ECG telemetry recording was used to assess arrhythmogenic effects.

RESULTS: Increase in heart rate caused APD shortening. This shortening had a significant K_{ATP} channel component at high heart rates and was diminished by glyburide. This glyburide-sensitive component was also identified under drug-induced APD prolongation in WT but not in Kir6.2-KO at higher but not slower heart rates indicating an energetic load-dependent control of APD by K_{ATP} channels. *In vivo* ECG telemetry in the absence of functional K_{ATP} channels revealed significantly higher vulnerability to drug-induced development of QT prolongation and polymorphic ventricular tachycardia (TdP).

CONCLUSION: K_{ATP} channels are critical in limiting drug-induced APD prolongation especially at high cardiac workload. This cardioprotective effect is counteracted by sulfonylureas. This study identifies K_{ATP} channels as potential therapeutic targets for the treatment of LQTS, and points to potentially harmful electrophysiological effects of sulfonylurea drugs.

OI-A-I

POPULATION PHARMACOKINETIC-PHARMACODYNAMIC (PK-PD) MODEL OF THE VASCULAR-DISRUPTING AGENT 5,6-DIMETHYLXANTHENE-4-ACETIC ACID (DMXAA) IN CANCER PATIENTS. J. Li, PhD, M. B. Jameson, MD, B. C. Baguley, MD, R. Pili, MD, S. D. Baker, PharmD, PhD, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Waikato Hospital, University of Auckland, Baltimore, MD.

BACKGROUND: DMXAA selectively disrupts established tumor blood vessels. The degree of DMXAA-induced tumor hemorrhagic necrosis is correlated with increased plasma 5-hydroxyindole-3-acetic acid (5-HIAA) (a metabolite of serotonin). DMXAA is being evaluated in phase II trials in combination with cytotoxics.

METHODS: The plasma DMXAA and 5-HIAA concentration data were obtained from 124 patients receiving DMXAA monotherapy as 20-min intravenous infusion weekly or every three weeks at doses ranging from 6 to 4900 mg/m² in three phase I trials. The PK

and PD data were analyzed by nonlinear mixed effect modeling with NONMEM program. Potential covariates including age, body weight, height, body surface area (BSA), sex, liver and kidney functions were screened with general additive model analysis and tested in the PK/PD model.

RESULTS: DMXAA concentration-time profiles were well described by a 3-compartment model with saturable elimination (Michaelis-Menten kinetic). BSA and sex were significant covariates on the volume of distribution of the central compartment (V1) and the maximum elimination rate (VM), respectively, accounting for 10 and 12% of interindividual variation in these parameters. Population estimates for VM, KM (concentration at which half VM is achieved), and V1 were $122 \times (1 + 0.502 \times (2-SEX))$ ($\mu\text{M/h}$) [SEX = 1 for males, 2 for females], 103 μM , and $8.15 \times (BSA/1.8)^{0.69}$ (L), respectively. Plasma DMXAA and 5-HIAA concentrations were simultaneously fitted by the effect compartment PK-PD model with population PK parameters fixed to the previously obtained values from the final PK model. The effect of DMXAA on plasma 5-HIAA was described by the stimulatory E_{max} model, where population estimates for baseline, E_{max} , and EC_{50} were 46.2 μM , 2.75-fold increase of the baseline value, and 659 μM , respectively.

CONCLUSIONS: DMXAA plasma disposition is characterized by a saturable elimination process. BSA-guided dosing is important. 5-HIAA can be used as a biomarker to guide dose selection. DMXAA doses of 1000 to 2000 mg/m², resulting in 1.5- to 1.8-fold increase of plasma 5-HIAA, are recommended for phase II/III studies.

OI-A-II

THE DOSE-RESPONSE RELATIONSHIP BETWEEN MANNITOL AND INTRACRANIAL PRESSURE IN TRAUMATIC BRAIN INJURY PATIENTS. M. D. Sorani, UCSF, San Francisco, CA.

BACKGROUND/AIMS: Brain edema, an increase in water content in the brain, plays an important role in the pathophysiology of traumatic brain injury (TBI). Edema can increase intracranial pressure (ICP) and lead to ischemia and death. Brain edema is often treated with intravenous hyperosmotic agents to draw water out of tissue and decrease ICP. Mannitol, an alcohol sugar, is the osmotic diuretic most commonly used, yet no definitive dose-response relationship has been established. The specific relationship is important since high doses increase serum osmolality and have been associated with renal failure, whereas low doses may be ineffectual. Our aim was to characterize the dose-response relationship between mannitol and ICP in TBI patients.

METHODS: In a retrospective study at an urban trauma center intensive care unit, we measured ICP using a continuous physiological monitoring system in 28 consecutive TBI patients who were given at least one bolus of mannitol. Twenty patients were given a total of 85 doses of 50 g of mannitol, and 18 patients were given 50 doses of 100 g. Some patients received both doses.

RESULTS: Average ICP was 22.0 ± 10.6 mm Hg at the time mannitol was administered, fell immediately after dosing, and continued falling for approximately 30 minutes to 15.7 ± 8.1 mm Hg across all patients. After 30 minutes, ICP in the 100 g group (15.6 ± 10.9 mm Hg) was similar to that in the 50 g group (15.7 ± 6.3 mm Hg). However at 100 minutes, ICP had increased in the 50 g group to nearly its initial value but was lower in the 100 g group (18.6 ± 7.6 v. 14.2 ± 6.7 mm Hg; $p = .001$). We plotted ICP decrease versus weight-adjusted dose and found a linear relationship ($y = 12.1x - 5.6$, $p = .003$). The relationship indicates that each additional 0.1 g/kg mannitol achieves an additional reduction of approximately 1.2 mm Hg in ICP. When we separately analyzed doses given when patient ICP was greater than or less than 20 mm Hg, we found that doses given when ICP was high resulted in 36% or 43% decreases in ICP respectively for doses of 50 g or 100 g, while doses given when ICP was low showed only 4% or 13% decreases in ICP respectively.