OIII-B-1

THE EXPRESSION OF THE HUMAN ORGANIC CATION TRANSPORTER hOCT1 IN MAMMARY GLAND. U. Dhillon, BSc,* J. Shenton,* V. Cook, MSc,* P. Harper, PhD,* J. Watson-Macdonell, RN, MSc,* and S. Ito, MD, Division of Clin Pharmacol and Toxicol, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada.

The transfer of drugs to nursing infants from mother's milk is of significant clinical importance. Recent studies have shown evidence of organic cation transport into human milk. A potential-dependent cation transporter (hOCT1) was recently found to be expressed in many human tissues including liver, kidney, intestine and placenta. We examined the expression of hOCT1 in a human mammary epithelial cell line MCF12A. Using RT-PCR we have detected mRNA for hOCT1 in this cell line. The digestion of the hOCT1 fragment with PstI has confirmed the identity of the PCR product. Functional analysis has revealed a time-dependent uptake of tetraethyl-ammonium (TEA) by MCF12A cells which is inhibitable by excess TEA. We have also found the expression of hOCT1 mRNA through RT-PCR in cells isolated from human milk obtained from lactating women. This is the first study to suggest that hOCT1 may be responsible for the transport of organic cations by the mammary gland epithelia into human milk.

(Supported by MRC.)

OIII-B-2

OATP AND P-GLYCOPROTEIN MEDIATE THE UPTAKE AND EXCRETION OF FEXOFENADINE. R.B. Kim, MD, M. Cvetkovic, MD,* M.F. Fromm, MD, B. Leake, BSc,* and G.R. Wilkinson, PhD, Div of Clin Pharmacol, Vanderbilt University, Nashville, TN.

Fexofenadine (Allegra™) is a non-sedating antihistamine that is eliminated essentially unchanged, primarily in the urine and feces. Accordingly, it was hypothesized that transport proteins, both for uptake and excretion, are possible determinants of fexofenadine disposition. Utilizing a recombinant vaccinia expression system, members of the organic anion transporting polypeptide (OATP) family, such as the human and rat OATP1, and rat OATP2, but not the bile acid transporter NTCP or the organic cation transporter OCT1, were found to mediate [^{14}C]-fexofenadine cellular uptake. *MDR1* gene encoded P-glycoprotein (P-gp), was identified as a fexofenadine efflux transporter, using the LLC-PK1 cells, a polarized epithelial cell line lacking P-gp, and the derivative cell line (L-MDR1), overexpressing P-gp. That is, fexofenadine transport was enhanced in the basal-to-apical direction in the L-MDR1, but not in the LLC-PK1 cells. In addition, oral and intravenous administration of [^{14}C]-fexofenadine to mice lacking the *mdr1a* gene resulted in 5- and 9-fold increases in fexofenadine plasma and brain levels at 4 hr respectively, compared to wildtype mice. Since OATP uptake transporters and the efflux transporter P-gp are co-localized in organs of importance to drug disposition, such as the liver and the kidney, coordinate activity of these transport processes provides an explanation for the, heretofore, unknown mechanism(s) involved in fexofenadine disposition.

OIII-B-3

LOPERAMIDE CAUSES RESPIRATORY DEPRESSION WHEN P-GP IS INHIBITED. A.J.M. Sadeque, PhD,* C. Wandel, MD, S. Shah,* A.J.J. Wood, MD, Div of Clinical Pharmacology, Vanderbilt Univ Sch of Med, Nashville, TN.

The antidiarrheal agent loperamide is a potent opioid yet clinically its effects are usually limited to the GI tract without the CNS effects and respiratory depression characteristic of other opiates. Because of evidence suggesting loperamide is a P-glycoprotein (P-gp) substrate, we postulated that inhibition of P-gp in the brain by quinidine would increase loperamide's CNS penetration and result in respiratory depression. Eight healthy volunteers received loperamide (16 mg) accompanied by either quinidine (600 mg once) or placebo in random order. Respiratory depression was evaluated by measuring the ventilatory response to increasing concentrations of CO_2. Ventilation was depressed when quinidine was given with loperamide compared to loperamide alone, resulting in significant reduction of the slope of the CO_2 response curve ($P<0.003$) and minute ventilation at a pCO_2 of 50 mm/Hg ($P<0.03$). Thus although loperamide is widely used without medical supervision as an antidiarrheal agent, inhibition of P-gp results in opiate CNS effects, so that the coadministration of drugs which inhibit P-gp may produce clinically significant drug interactions by a novel mechanism.

OIII-B-4

GRAPEFRUIT JUICE EXERTS STIMULATORY EFFECTS ON P-GLYCOPROTEIN. A. Soldner, PhD,*[1] U. Christians, MD, PhD,*[1] M. Susanto, BSc,*[1] V.J. Wacher, PhD,*[2] J.A. Silverman, PhD,*[2] and L.Z. Benet, PhD,[1] Department of Biopharmaceutical Sciences, UCSF,[1] San Francisco, and AvMax Inc,[2] Berkeley, CA.

Grapefruit juice (GJ) is known to increase the bioavailability of many CYP3A-substrates by inhibiting intestinal phase-I metabolism. However, the magnitude of AUC increase is often insignificant and highly variable. Since we earlier suggested that CYP3A and P-glycoprotein (p-gp) form a concerted barrier to drug absorption, we investigated the role of p-gp in GJ-drug interactions. The transcellular bidirectional flux of drugs that are (i) CYP3A- and/or p-gp substrates (Vinblastine [Vin], Cyclosporin A [CsA], Digoxin [Dig], Fexofenadine [Fex]) or that are (ii) only CYP3A-substrates (Felodipine [Fel], Nifedipine [Nif]) was evaluated across MDR1-MDCK and Caco-2 monolayers in the presence and absence of GJ, verifying monolayer integrity at all times. While both apical-to-basal (A-B) and basal-to-apical (B-A) fluxes of all CYP3A/p-gp substrates tested were increased in the presence of GJ, the resulting net secretion (B-A/A-B) was in all cases significantly greater with GJ than control (in MDR1-MDCK: Vin, 99.2 vs 9.0; CsA, 28.8 vs 10.2; Dig, 22.7 vs 14.6, Fex, 18.6 vs 11.7). In contrast, no GJ flux effect was observed with Fel and Nif, substrates of CYP3A, but not of p-gp (1.2 vs 0.84 and 1.5 vs 2.0). In conclusion, GJ has been found to significantly stimulate p-gp-mediated secretory efflux of drugs that are substrates of CYP3A and/or p-gp, thereby partially counteracting the CYP3A-inhibitory effects of GJ.