

Cryopreserved human enterocytes

**Human enterocytes as an in vitro model for the evaluation of intestinal drug metabolism:
Characterization of drug metabolizing enzyme activities of cryopreserved human enterocytes from
twenty four donors**

Ming-Chih David Ho, Nicholas Ring, Kirsten Amaral, Utkarsh Doshi, and Albert P. Li

Running title: Cryopreserved human enterocytes

Corresponding author: Albert P. Li, 9221 Rumsey Road, Suite 8, Columbia, MD 21045; email:

lialbert1@invitroadmet.com

Number of text pages: 15

Number of tables: 4

Number of figures: 1

Number of references: 32

Abstract: 211 words

Introduction: 359 words

Discussion: 909 words

Nonstandard Abbreviations:

CERM™: Cryopreserved enterocytes recovery medium

HQM™: Hepatocyte/enterocyte incubation medium

Abstract

We report here successful isolation and cryopreservation of enterocytes from human small intestine. The enterocytes were isolated by enzyme digestion of the intestinal lumen followed by partial purification via differential centrifugation. The enterocytes were cryopreserved directly after isolation without culturing to maximize retention of *in vivo* drug metabolizing enzyme activities. Post-thaw viability of the cryopreserved enterocytes was consistently over 80% based on trypan blue exclusion. Cryopreserved enterocytes pooled from 8 donors (4 male and 4 female) were evaluated for their metabolism of 14 pathway-selective substrates: CYP1A2 (phenacetin hydroxylation), CYP2A6 (coumarin 7-hydroxylation), CYP2B6 (bupropion hydroxylation), CYP2C8 (paclitaxel 6 α -hydroxylation), CYP2C9 (diclofenac 4-hydroxylation), CYP2C19 (*s*-mephenytoin 4-hydroxylation), CYP2D6 (dextromethorphan hydroxylation), CYP2E1 (chlorzoxazone 6-hydroxylation), CYP3A4 (midazolam 1'-hydroxylation and testosterone 6 β -hydroxylation), CYP2J2 (astemizole O-demethylation), UDP-glucuronosyltransferase (UGT; 7-hydroxycoumarin glucuronidation), sulfotransferase (SULT; 7-hydroxycoumarin sulfation), and carboxylesterase 2 (CES2; irinotecan hydrolysis) activities. Quantifiable activities were observed for CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP3A4, CYPJ2, CES2, UGT and SULT, but not for CYP1A2, CYP2A6, CYP2B6 and CYP2D6. Enterocytes from all 24 donors were then individually evaluated for the quantifiable drug metabolism pathways. All demonstrated quantifiable activities with the expected individual variations. Our results suggest that cryopreserved human enterocytes represent a physiologically relevant and convenient *in vitro* experimental system for the evaluation of intestinal metabolism, akin to cryopreserved human hepatocytes for hepatic metabolism.

Introduction

Intestinal metabolism has been reported to be responsible for the low bioavailability of approximately 50% of orally-administered drugs (Kaminsky and Zhang 2003; Thummel 2007; Wacher et al. 2001; Watkins 1992). In vitro evaluation of intestinal drug metabolism therefore represents an experimental approach that can be applied to guide the development of drugs with acceptable oral bioavailability. The commonly used in vitro model of intestinal mucosal epithelium, the colon adenocarcinoma Caco2 cell line, while useful in defining permeability and P-gp efflux, is generally not considered appropriate for the evaluation of enteric drug metabolism due to the low basal level of drug metabolizing enzyme activity (Nakamura et al. 2002; Prueksaritanont et al. 1996). To overcome this deficiency, Caco2 cells have been transfected with human P450 isoforms, especially CYP3A4, for the evaluation of drug bioavailability in the presence of P450 metabolism (Crespi et al. 1996; Kublbeck et al. 2016). However, this approach is far from representative of intestinal metabolism which is known to involve multiple pathways, including various P450 isoforms and non-P450 drug metabolizing enzymes (Nakamura et al. 2016; Paine et al. 2006).

Current in vitro experimental models of human intestinal metabolism include human intestinal microsomes (Galetin et al. 2008; Kolars et al. 1992), precision cut intestinal slices (van de Kerkhof et al. 2008), and freshly isolated enterocytes (Bader et al. 2000; Bonnefille et al. 2011; Hansen et al. 2000; Zhang et al. 2003). Of these systems, primary enterocytes may represent the most physiologically relevant model, akin to primary hepatocytes for hepatic metabolism. Intact enterocytes possess key cellular properties which are key to the assessment of in vivo events including an intact plasma membrane to allow modeling of membrane permeability, uptake and efflux drug transporters, as well as

Cryopreserved human enterocytes

complete and uninterrupted drug metabolizing enzyme pathways and cofactors for both phase I oxidation and phase II conjugation.

In our laboratory, we embarked upon the isolation and cryopreservation of enterocytes with the goal of developing a physiologically relevant experimental model for the evaluation of intestinal uptake, metabolism and efflux. We report here our success in the isolation and cryopreservation of human enterocytes to retain viability and drug metabolism enzyme activities.

Materials and Methods:

Chemicals. Astemizole, irinotecan hydrochloride, dextrorphan tartrate, diclofenac sodium salt, 7-ethyl-10-hydroxycamptothecin (SN38), 4-hydroxydiclofenac, *s*-mephenytoin, 4-hydroxymephenytoin, paclitaxel, and testosterone were purchased from Cayman Chemical (Ann Arbor, MI). 7-Hydroxycoumarin was purchased from Chem Service (West Chester, Pennsylvania). Bupropion hydrochloride was obtained from AK Scientific (Union City, CA). 7-Hydroxycoumarin sulfate potassium salt was obtained from Santa Cruz Biotechnology (Dallas, Texas). Chlorzoxazone, coumarin, dextromethorphan hydrobromide, 6 β -hydroxytestosterone, 7-hydroxycoumarin β -D-glucuronide sodium salt, (2*S*, 3*S*)-hydroxy bupropion hydrochloride, 7-ethoxycoumarin, and phenacetin were purchased from Sigma-Aldrich (St. Louis, MO). 6-Hydroxychlorzoxazone, 6 α -hydroxypaclitaxel, midazolam, 1'-hydroxymidazolam, *O*-desmethyl astemizole, and 4-hydroxy-*S*-mephenytoin were obtained from Toronto Research Chemicals (Toronto, Canada).

Human intestine. Human intestines from multiple donors were obtained from the International Institute for the Advancement of Medicine (IIAM, Exton, PA).

Enterocyte isolation and cryopreservation. Isolation of enterocytes from human intestines was performed via enzymatic digestion of the intestinal lumen based on procedures previously reported for porcine intestines (Bader et al. 2000; Hansen et al. 2000). The following lengths (post-pyloric sphincter) were used to aid the identification of the various regions of the small intestine for enterocyte isolation: duodenum: 26 centimeters (9.84 inches), jejunum: 2.5 meters (8.2 feet) and ileum: 3.5 meters. The intestines were recovered with a warm ischemic time of less than 15 minutes, and shipped to our laboratory on wet ice in University of Wisconsin Preservation Solution, with a cold ischemic time of less than 24 hours.

Cryopreserved human enterocytes

Upon receipt of the small intestines, adipose tissue associated with intestines was removed by dissection. The intestinal lumen was washed rapidly with cold calcium and magnesium free Hank's Balanced Salt Solution to remove intestinal contents followed by digestion with an isotonic buffer containing 0.25 mg/mL of type I collagenase (Sigma-Aldrich). The cells released from the intestinal lumen were sieved to remove relatively large cell clusters followed by partial purification by differential centrifugation (100xg, 20 minutes). The enterocytes were quantified and cryopreserved immediately after isolation using a programmable liquid nitrogen cell freezer and stored in the vapor phase of liquid nitrogen maintained at <-150°C.

Recovery of cryopreserved enterocytes. Cryopreserved enterocytes (In Vitro ADMET Laboratories, Columbia, MD) were thawed in a 37°C water bath for approximately 2 min and transferred by pouring into a 50 ml conical of Cryopreserved Enterocyte Recovery Medium, (CERM™, In Vitro ADMET Laboratories, Columbia, MD) that was pre-warmed in a 37°C water bath. The thawed enterocytes were recovered by centrifugation at 100 x g for 10 min at room temperature. After centrifugation, the supernatant was removed by decanting. A volume of 250 µl of 4°C Hepatocyte/Enterocyte Incubation Medium, (HQM™, In Vitro ADMET Laboratories, Columbia, MD) was added to the intact pellet of enterocytes at the bottom of the conical tube followed by gently agitation to reconstitute an enterocyte suspension. Viability and yield were quantified in a hemacytometer based on trypan blue dye exclusion (Sigma-Aldrich).

Measurement of enterocyte diameter. Photomicrographs of the enterocytes were taken using a phase contrast photomicroscope. The photomicrographs were printed and the diameters were measured and corrected for the magnification factor. Results (µm) are expressed as mean and standard deviation values of 50 randomly chosen cells from each enterocyte lot.

Incubation of enterocytes with drug metabolizing enzyme substrates. DME substrate incubations were performed in a cell culture incubator maintained at 37°C with a humidified atmosphere of 5% CO₂. Enterocyte cell density was adjusted to 3 x 10⁶ cells/ml in HQM™. Aliquots of 50 µl cell suspension were added to individual wells (150,000 viable enterocytes/well) of a 96-well plate for the evaluation of drug metabolism activities. After cell addition, the 96-well plate was pre-warmed in the incubator for 15 min, followed by the addition of 50 µl of pre-warmed (37°C) HQM™ containing DME substrates at 2x final concentration and incubated for 2 hrs. The final incubation mixture in each well therefore had a volume of 100 µl, with a cell density of 1.5 x 10⁶ cells/ml. Substrates for the multiple drug metabolism pathways evaluated are shown in Table 1. Metabolism was terminated in each well by the addition of 100 µl acetonitrile. The final incubation samples were stored at -80°C for the subsequent LC/MS-MS analysis.

LC/MS-MS quantification of metabolite formation. Upon thawing, an aliquot of 100 µl of acetonitrile containing an internal standard of 250 nM tolbutamide was added to each sample. All samples were centrifuged at 13,000 rpm for 5 minutes. An aliquot of 100 µL of supernatant from each was transferred to a 96 well plate and was diluted with 200 µL of deionized water for LC/MS-MS analysis using an API 4000 QTRAP mass spectrometer with an electrospray ionization source (AB SCIEX, Framingham, MA) connected to Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA). An Agilent Zorbax Eclipse Plus C18 column (4.6 x 75 mm i.d., 3.5 µm; Agilent Technologies, Santa Clara, CA) at a flow rate of 1 mL/min was used for the chromatography separation. The mobile phase consisted of 0.1 % formic acid in acetonitrile (A) and 0.1 % formic acid in water (B). The gradient for the positive ion mode operation was programmed as: 0 to 2.5 min, increase B from 5 to 95%; 2.5 to 3.5 min, 95% B; 3.5 to 3.6 min, decrease B to 5%; run-time 5 min. The gradient program for the negative ion mode was: 0 to 3 min,

Cryopreserved human enterocytes

increase B from 5 to 95%; 3 to 4 min, 95% B; 4 to 4.2 min, decrease B to 5%; run-time 6 min. Data acquisition and data procession were performed with the software Analyst 1.6.2 (AB SCIEX, Framingham, MA). Standard assays of the metabolites were performed in LC/MS-MS MRM mode, monitoring the mass transitions (parent to daughter ion). The metabolism substrates used, identities of the metabolites quantified, and LC/MS-MS parameters are shown in Table 1.

Results

Donor demographics: Enterocytes were isolated and cryopreserved from 25 donors. Age, gender, and ethnicity of the donors are shown in Table 2.

Morphology, size, viability and yield of cryopreserved human enterocytes. The morphology of cryopreserved human enterocytes immediately after recovery is shown in Figure 1. The enterocytes consisted of either single cells or small cell clusters, with a rounded morphology typical of mammalian cells in suspension. The average post-thaw viability of human enterocytes for the 25 donors was $84.7 \pm 4.7\%$ with a range of 78% to 95%, with an average diameter of $14.9 \pm 1.0 \mu\text{m}$ (Table 2).

Drug metabolizing enzyme activities.

Metabolism of 14 substrates using pooled human enterocytes: Human enterocytes from 4 male and 4 female donors were combined (pooled) and incubated with 14 pathway-selective substrates for the evaluation of their drug metabolism potential. Results are shown in Table 3.

Individual variations in drug metabolizing enzyme activities: Enterocytes from 24 donors were evaluated for CYP2C9 (diclofenac 4-hydroxylation), CYP2C19 (*s*-mephenytoin 4-hydroxylation), CYP3A4 (midazolam 1'-hydroxylation and testosterone 6 β -hydroxylation), CYP2J2 (astemizole O-demethylation), CES (irinotecan hydrolysis), UGT (7-hydroxycoumarin glucuronidation), and SULT (7-hydroxycoumarin sulfation) activities. Results are shown in Table 4.

Discussion

Intestinal uptake, efflux, and metabolism are the three key determinants of bioavailability of orally administered drugs. Enterocytes isolated from the small intestines represent an ideal model to evaluate these determinants. Successful isolation of primary enterocytes to retain drug metabolizing enzyme activities have been previously reported using a variety of procedures including the following: from mice via EDTA perfusion (Zhang et al. 2003), from pigs via collagenase digestion (Bader et al. 2000; Hansen et al. 2000), and from humans via mechanical separation (Chougule et al. 2012). Our report here represents the first to report successful cryopreservation of primary enterocytes isolates from humans to retain viability and drug metabolizing enzyme activities. Using enterocytes pooled from 4 male and 4 female donors, quantifiable metabolite formation was observed for CYP2C8 (paclitaxel 6 α -hydroxylation), CYP2C9 (diclofenac 4-hydroxylation), CYP2C19 (*s*-mephenytoin 4-hydroxylation), CYP2E1 (chlorzoxazone 6-hydroxylation), CYP3A4 (midazolam 1'-hydroxylation and testosterone 6 β -hydroxylation), CYP2J2 (astemizole O-demethylation), CES (irinotecan hydrolysis), UGT (7-hydroxycoumarin glucuronidation), and SULT (7-hydroxycoumarin sulfation). Undetectable activities were observed for the following drug metabolizing enzymes: CYP1A2 (phenacetin hydroxylation), CYP2A6 (coumarin 7-hydroxylation), CYP2B6 (bupropion hydroxylation), and CYP2D6 (dextromethorphan hydroxylation). Ranking of activities in descending order is as follows:

CYP3A4 (testosterone) > SULT > UGT > CYP3A4 (midazolam) > CYP2C9 > CYP2J2 > CYP2C19 = CYP2E1 \geq CES2 \geq CYP2C8

Our ranking of the quantifiable drug metabolizing enzymes in enterocytes are consistent with that reported by others for P450 (Paine et al. 2006; Xie et al. 2016), UGT (Bock 2016; Radomska-Pandya et al. 1998), and SULT (Chen et al. 2003; Teubner et al. 2007), with CYP3A4 and UGT are believed to be the

Cryopreserved human enterocytes

most important drug metabolizing enzymes responsible for metabolism-dependent enteric drug bioavailability. One interesting observation is that lack of significant CYP2D6 activity in the intestines, as this P450 isoform is known to be specifically responsible for hepatic metabolism of a large number of commonly used human drugs, especially for antipsychotic drugs (Vandel et al. 1999). The lack of CYP2D6 activities is consistent with the findings with oxycodone metabolism by intestinal mucosal microsomes where the CYP3A4 mediated *N*-demethylation, but not the CYP2D6-mediated *O*-demethylation was observed (Lalovic et al. 2004).

Based on the results with the pooled enterocytes, enterocytes from 24 donors were evaluated to estimate the extent of inter-individual variations in enteric drug metabolism. The 24 donors represent the enterocytes that we successfully isolated and cryopreserved at the time of the preparation of this manuscript. The results confirmed that enterocytes possess CYP2C9, CYP2C19, CYP3A4, CYP2J2, CES2, UGT and SULT activities, with substantial inter-individual differences. The range of activities, expressed as pmol/min/million enterocytes, for the various drug metabolizing enzymes are: CYP2C9: 0.03 (HE3011) – 7.93 (HH3034); CYP2C19: 0.01 (HE3011) – 1.13 (HE3033); CYP3A4 (midazolam 1'-hydroxylation): 0.09 (HE3011) – 4.35 (HH3034); CYP3A4 (testosterone 6 β -hydroxylation): 2.60 (HE3011) – 45.2 (HE3034); CYP2J2: 0.19 (HE3021) – 1.98 (HE3034); CES2: 0.05 (HE3030) – 0.60 (HE3009); UGT: 1.01 (HE3011) – 122.6 (HE3034); SULT: 0.79 (HE3031) – 22.0 (HE3040). An interesting observation is that one of the enterocyte lots, HE3034 (from a 50 year old female Caucasian), was found to have UGT activity of 122.6 pmol/min/million enterocytes, approximately 12 fold of the average of the values for enterocytes from the other 23 donors (ranging from 1.01 to 29.6 pmol/min/million enterocytes) of 9.7 pmol/min/million enterocytes. This same donor also had highest CYP3A4 and CYP2J2 activities. In contrary, HE 3011 (from a 53 year old female Caucasian), had the lowest activities for CYP2C9, CYP2C19, CYP3A4, and UGT activities. Individual differences in intestinal metabolism is a potential determinant in

Cryopreserved human enterocytes

individual differences in bioavailability for orally administered drugs (Jamei et al. 2009). Our results suggest that enterocytes from individuals with different drug metabolizing enzyme activities may be used to evaluate this important drug property. However, It is to be added that cryopreservation may lead to attenuation of drug metabolizing enzyme activities. This possibility is being investigated in our laboratory. For now, our data definitively demonstrate that the cryopreserved enterocytes were active in the drug metabolizing enzyme activities evaluated. Whether the cryopreserved enterocyte reflect in vivo activities is yet to be determined.

Besides the metabolic activities shown here, we have quantified gene expression of the enterocytes from the multiple donors and demonstrated that the cells consistently express enterocyte-specific markers, P450 isoforms, uptake and efflux transporters (manuscript in preparation).

Cryopreserved enterocytes may represent the “gold standard” for enteric metabolism studies, as do human hepatocytes for hepatic metabolism (Fabre et al. 1990; Gomez-Lechon et al. 2004; Li 2010), for similar reasons. An intact plasma membrane with active transporters allows the modeling of drug permeability. Complete, uninterrupted drug metabolizing enzyme systems allow simultaneous evaluation of multiple metabolic pathways. CYP450 and phase II DMEs and their cofactors exist at physiological concentrations in enterocytes, which minimizes experimental artifacts such as ubiquitous CYP450 protein binding observed in human liver microsomes. Finally, cytosolic proteins in enterocytes allow modeling of intracellular protein binding. Cryopreserved enterocytes may be used routinely in drug development to allow the optimization of drug candidates with the most appropriate enteric metabolic properties. Two major potential applications of cryopreserved human enterocytes are: 1. investigation of the role of enteric metabolism on bioavailability of orally administered drugs; and, 2.

Cryopreserved human enterocytes

enteric drug-drug and food-drug interactions which occurs specifically in the intestine but not in the liver, as exemplified by the findings with grapefruit juice on intestinal drug metabolism (Holmberg et al. 2014), uptake (Shirasaka et al. 2013) and efflux (Wang et al. 2001). A major focus of our laboratory currently is the development of experimental approaches using cryopreserved human enterocytes to evaluate key enteric drug properties including metabolic clearance, metabolite profiling, transporter-mediated uptake and efflux, P450 inhibition and induction, and enterotoxicity.

Authorship Contributions

Participated in research design: Li, A.P., Ho, D., Doshi, U.

Conducted experiments: Ho, D., Amaral, K., Ring, N., and Doshi, U.

Performed data analysis: Ho, D., Ring, N., Doshi, U., and Li, A.P.

Manuscript preparation: Li, A. P., Ho, D., Ring, N., Amaral, K., Doshi, U.

References

- Bader A, Hansen T, Kirchner G, Allmeling C, Haverich A, and Borlak JT. 2000. Primary porcine enterocyte and hepatocyte cultures to study drug oxidation reactions. *Br J Pharmacol* 129(2):331-342.
- Bock KW. 2016. The UDP-glycosyltransferase (UGT) superfamily expressed in humans, insects and plants: Animal-plant arms-race and co-evolution. *Biochem Pharmacol* 99:11-17.
- Bonnefille P, Sezgin-Bayindir Z, Belkhefja H, Arellano C, Gandia P, Woodley J, and Houin G. 2011. The use of isolated enterocytes to study Phase I intestinal drug metabolism: validation with rat and pig intestine. *Fundam Clin Pharmacol* 25(1):104-114.
- Chen G, Zhang D, Jing N, Yin S, Falany CN, and Radomska-Pandya A. 2003. Human gastrointestinal sulfotransferases: identification and distribution. *Toxicol Appl Pharmacol* 187(3):186-197.
- Chougule P, Herlenius G, Hernandez NM, Patil PB, Xu B, and Sumitran-Holgersson S. 2012. Isolation and characterization of human primary enterocytes from small intestine using a novel method. *Scand J Gastroenterol* 47(11):1334-1343.
- Crespi CL, Penman BW, and Hu M. 1996. Development of Caco-2 cells expressing high levels of cDNA-derived cytochrome P4503A4. *Pharm Res* 13(11):1635-1641.
- Fabre G, Combalbert J, Berger Y, and Cano JP. 1990. Human hepatocytes as a key in vitro model to improve preclinical drug development. *Eur J Drug Metab Pharmacokinet* 15(2):165-171.
- Galetin A, Gertz M, and Houston JB. 2008. Potential role of intestinal first-pass metabolism in the prediction of drug-drug interactions. *Expert Opin Drug Metab Toxicol* 4(7):909-922.
- Gomez-Lechon MJ, Donato MT, Castell JV, and Jover R. 2004. Human hepatocytes in primary culture: the choice to investigate drug metabolism in man. *Curr Drug Metab* 5(5):443-462.
- Hansen T, Borlak J, and Bader A. 2000. Cytochrome P450 enzyme activity and protein expression in primary porcine enterocyte and hepatocyte cultures. *Xenobiotica* 30(1):27-46.
- Holmberg MT, Tornio A, Neuvonen M, Neuvonen PJ, Backman JT, and Niemi M. 2014. Grapefruit juice inhibits the metabolic activation of clopidogrel. *Clin Pharmacol Ther* 95(3):307-313.
- Jamei M, Turner D, Yang J, Neuhoff S, Polak S, Rostami-Hodjegan A, and Tucker G. 2009. Population-based mechanistic prediction of oral drug absorption. *AAPS J* 11(2):225-237.
- Kaminsky LS, and Zhang QY. 2003. The small intestine as a xenobiotic-metabolizing organ. *Drug Metab Dispos* 31(12):1520-1525.
- Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, and Watkins PB. 1992. Identification of rifampin-inducible P450III A4 (CYP3A4) in human small bowel enterocytes. *J Clin Invest* 90(5):1871-1878.
- Kublbeck J, Hakkarainen JJ, Petsalo A, Vellonen KS, Tolonen A, Reponen P, Forsberg MM, and Honkakoski P. 2016. Genetically Modified Caco-2 Cells With Improved Cytochrome P450 Metabolic Capacity. *J Pharm Sci* 105(2):941-949.
- Lalovic B, Phillips B, Risler LL, Howald W, and Shen DD. 2004. Quantitative contribution of CYP2D6 and CYP3A to oxycodone metabolism in human liver and intestinal microsomes. *Drug Metab Dispos* 32(4):447-454.
- Li AP. 2010. Evaluation of drug metabolism, drug-drug interactions, and in vitro hepatotoxicity with cryopreserved human hepatocytes. *Methods Mol Biol* 640:281-294.
- Nakamura K, Hirayama-Kurogi M, Ito S, Kuno T, Yoneyama T, Obuchi W, Terasaki T, and Ohtsuki S. 2016. Large-scale multiplex absolute protein quantification of drug-metabolizing enzymes and transporters in human intestine, liver, and kidney microsomes by SWATH-MS: Comparison with MRM/SRM and HR-MRM/PRM. *Proteomics* 16(15-16):2106-2117.
- Nakamura T, Sakaeda T, Ohmoto N, Tamura T, Aoyama N, Shirakawa T, Kamigaki T, Kim KI, Kim SR, Kuroda Y et al. . 2002. Real-time quantitative polymerase chain reaction for MDR1, MRP1, MRP2, and CYP3A-mRNA levels in Caco-2 cell lines, human duodenal enterocytes, normal colorectal tissues, and colorectal adenocarcinomas. *Drug Metab Dispos* 30(1):4-6.

- Paine MF, Hart HL, Ludington SS, Haining RL, Rettie AE, and Zeldin DC. 2006. The human intestinal cytochrome P450 "pie". *Drug Metab Dispos* 34(5):880-886.
- Prueksaritanont T, Gorham LM, Hochman JH, Tran LO, and Vyas KP. 1996. Comparative studies of drug-metabolizing enzymes in dog, monkey, and human small intestines, and in Caco-2 cells. *Drug Metab Dispos* 24(6):634-642.
- Radomska-Pandya A, Little JM, Pandya JT, Tephly TR, King CD, Barone GW, and Raufman JP. 1998. UDP-glucuronosyltransferases in human intestinal mucosa. *Biochim Biophys Acta* 1394(2-3):199-208.
- Shirasaka Y, Shichiri M, Mori T, Nakanishi T, and Tamai I. 2013. Major active components in grapefruit, orange, and apple juices responsible for OATP2B1-mediated drug interactions. *J Pharm Sci* 102(1):280-288.
- Teubner W, Meinel W, Florian S, Kretzschmar M, and Glatt H. 2007. Identification and localization of soluble sulfotransferases in the human gastrointestinal tract. *Biochem J* 404(2):207-215.
- Thummel KE. 2007. Gut instincts: CYP3A4 and intestinal drug metabolism. *J Clin Invest* 117(11):3173-3176.
- van de Kerkhof EG, de Graaf IA, Ungell AL, and Groothuis GM. 2008. Induction of metabolism and transport in human intestine: validation of precision-cut slices as a tool to study induction of drug metabolism in human intestine in vitro. *Drug Metab Dispos* 36(3):604-613.
- Vandel P, Haffen E, Vandel S, Bonin B, Nezelof S, Sechter D, Broly F, Bizouard P, and Dalery J. 1999. Drug extrapyramidal side effects. CYP2D6 genotypes and phenotypes. *Eur J Clin Pharmacol* 55(9):659-665.
- Wacher VJ, Salphati L, and Benet LZ. 2001. Active secretion and enterocytic drug metabolism barriers to drug absorption. *Adv Drug Deliv Rev* 46(1-3):89-102.
- Wang EJ, Casciano CN, Clement RP, and Johnson WW. 2001. Inhibition of P-glycoprotein transport function by grapefruit juice psoralen. *Pharm Res* 18(4):432-438.
- Watkins PB. 1992. Drug metabolism by cytochromes P450 in the liver and small bowel. *Gastroenterol Clin North Am* 21(3):511-526.
- Xie F, Ding X, and Zhang QY. 2016. An update on the role of intestinal cytochrome P450 enzymes in drug disposition. *Acta Pharm Sin B* 6(5):374-383.
- Zhang QY, Dunbar D, and Kaminsky LS. 2003. Characterization of mouse small intestinal cytochrome P450 expression. *Drug Metab Dispos* 31(11):1346-1351.

Figure Legend

Figure 1: Phase contrast microscopy of cryopreserved and thawed human enterocytes. Enterocytes from HE3005, a 23 year old male Caucasian were shown. The cells exhibited rounded cell morphology with uniform cell size. The enterocytes were mainly single cells with some cell aggregates.

Cryopreserved human enterocytes

Table 1: Metabolic pathways, substrates, metabolites and LC/MS-MS parameters for the quantification of the drug metabolizing enzyme activities of cryopreserved human enterocytes. Tolbutamide was used as an internal standard with the MRM (Mass Transition Monitoring) at m/z 271.2 to 91.3 and m/z 269.1 to 105.9 for positive mode and negative mode, respectively.

Metabolic Pathway	Pathway-selective Substrate (μM)	Marker Metabolite	Ion Mode Application	Mass Transitions Monitoring
CYP1A2	Phenacetin (100)	Acetaminophen	Positive	m/z 152.1 to 109.9
CYP2A6	Coumarin (50)	7-Hydroxycoumarin	Negative	m/z 161.0 to 132.9
CYP2B6	Bupropion (500)	Hydroxybupropion	Positive	m/z 250.1 to 130.1
CYP2C8	Paclitaxel (20)	6 α -Hydroxypaclitaxel	Positive	m/z 870.4 to 525.2
CYP2C9	Diclofenac (25)	4-Hydroxydiclofenac	Negative	m/z 309.8 to 265.9
CYP2C19	S-Mephenytoin (250)	4-Hydroxy-S-Mephenytoin	Positive	m/z 235.2 to 150.0
CYP2D6	Dextromethorphan (15)	Dextrophan	Positive	m/z 258.1 to 157.1
CYP2E1	Chlorzoxazone (250)	6-Hydroxychlorzoxazone	Negative	m/z 183.9 to 119.8
CYP3A4-1	Midazolam (20)	1'-Hydroxymidazolam	Positive	m/z 342.1 to 203.1
CYP3A4-2	Testosterone (200)	6 β -Hydroxytestosterone	Positive	m/z 305.2 to 269.1
CYP2J2	Astemizole (50)	O-Demethylastemizole	Positive	m/z 445.0 to 204.2
CES2	Irinotecan (50)	SN38	Positive	m/z 393.0 to 349.3
UGT	7-Hydroxycoumarin (100)	7-Hydroxycoumarin Glucuronide	Negative	m/z 336.9 to 160.9
SULT	7-Hydroxycoumarin (100)	7-Hydroxycoumarin Sulfate	Negative	m/z 240.9 to 161.0

Cryopreserved human enterocytes

Table 2: Demographics, viability, yield and cellular diameter of human enterocytes from 25 individual donors (BMI: Body mass index).

Lot Number	Gender	Ethnicity	Age (years)	BMI	Viability (%)	Yield (million)	Cellular Diameter (µm)	Region
HE3005	Male	Caucasian	23	25	88	2.8	15.6	Duodenum
HE3006	Female	Caucasian	44	32.7	78	0.9	15.5	Duodenum
HE3007	Male	Hispanic	43	30.3	80	1.5	14.3	Duodenum
HE3008	Male	Caucasian	18	29.5	84	2.7	15	Duodenum
HE3009	Female	Caucasian	44	48.3	89	2.8	15	Duodenum
HE3010	Male	Caucasian	47	31.1	85	1.5	14.2	Duodenum
HE3011	Female	Caucasian	50	24.2	79	1.2	15.4	Duodenum
HE3013	Female	African American	57	33.0	75	1	14.8	Duodenum
HE3014	Male	African American	49	24.1	89	0.7	14.5	Duodenum
HE3015	Male	Caucasian	24	26.1	86	2.4	14.8	Duodenum
HE3016	Male	African American	32	23.4	89	1.4	15.4	Duodenum
HE3019	Male	Caucasian	61	30	89	0.7	15.4	Ileum
HE3020	Male	Caucasian	25	24.8	89	1.2	14.6	Duodenum
HE3021	Male	African American	60	20.7	88	0.7	12.9	Duodenum
HE3027	Female	Caucasian	53	36.5	85	1.6	14.4	Duodenum
HE3028	Male	African American	34	30.6	86	1.3	15.3	Duodenum
HE3029	Male	Caucasian	41	34.6	85	1.7	13.4	Duodenum
HE3030	Male	African American	38	21.1	78	0.7	14.5	Duodenum and jejunum
HE3031	Female	Caucasian	49	25.5	86	1	12.5	Duodenum
HE3032	Female	Caucasian	48	27.1	78	1.7	15.3	Duodenum
HE3033	Male	Hispanic	32	21	85	3.7	15.2	Duodenum and jejunum
HE3034	Female	Caucasian	53	18.8	95	3.5	17.2	Jujenum
HE3040	Male	African American	38	21.1	86	2.5	15.6	Small Intestine
HE3042	Male	Caucasian	60	22.8	85	2	16.2	Duodenum and jejunum
HE3043	Male	Caucasian	43	26.3	81	2	15	Duodenum and jejunum

Table 3. Drug metabolizing enzyme activities of cryopreserved human enterocytes. Enterocytes from 4 male (HE3005, HE3007, HE3008, HE3010) and 4 females (HE3006, HE3009, HE3011, HE3013) were pooled and used in the study. The drug metabolizing enzyme pathways evaluated were: CYP1A2 (phenacetin hydroxylation), CYP2A6 (coumarin 7-hydroxylation), CYP2B6 (bupropion hydroxylation), CYP2C8 (paclitaxel 6 α -hydroxylation), CYP2C9 (diclofenac 4-hydroxylation), CYP2C19 (s-mephenytoin 4-hydroxylation), CYP2D6 (dextromethorphan hydroxylation), CYP2E1 (chlorzoxazone 6-hydroxylation), CYP3A4 (midazolam 1'-hydroxylation (CYP3A4M) and testosterone 6 β -hydroxylation (CYP3A4T)), CYP2J2 (astemizole O-demethylation), UDP-glucuronosyltransferase (UGT; 7-hydroxycoumarin glucuronidation), sulfotransferase (SULT; 7-hydroxycoumarin sulfation), and carboxylesterase 2 (CES2; irinotecan hydrolysis) activities. Results are mean and standard deviation (sd) of triplicate determinations. Activities for CYP1A2, CYP2A6, CYP2B6, and CYP2D6 were below quantifiable levels (BQL). NA: Not applicable.

Drug Metabolizing Enzyme Pathway	Specific Activity (pmol/min/million enterocytes)	
	mean	sd
CYP1A2	BQL	NA
CYP2A6	BQL	NA
CYP2B6	BQL	NA
CYP2C8	0.2	0
CYP2C9	2.0	0.2
CYP2C19	0.4	0.1
CYP2D6	BQL	NA
CYP2E1	0.4	0.1
CYP3A4M	2.8	0.5
CYP3A4T	19.2	5.8
CYP2J2	1.6	0.1
CES2	0.3	0
UGT	4.5	0.3
SULT	11.4	0.7

Cryopreserved human enterocytes

Table 4. Drug metabolizing enzyme activities of cryopreserved human enterocytes from 24 donors. The mathematical average (mean) and standard deviation (sd) of the 24 donors are calculated from the results of each individual donor. The drug metabolizing enzyme pathways evaluated were: CYP2C9 (diclofenac 4-hydroxylation), CYP2C19 (s-mephenytoin 4-hydroxylation), CYP3A4 (midazolam 1'-hydroxylation (CYP3A4M) and testosterone 6 β -hydroxylation (CYP3A4T)), CYP2J2 (astemizole O-demethylation), CES (irinotecan hydrolysis), UGT (7-hydroxycoumarin glucuronidation), and SULT (7-hydroxycoumarin sulfation) activities. Mean and standard deviation (sd) values of the activities of the 24 donors are shown at the bottom of the table. ND: Not determined.

Lot Number	Activity (pmol/min/million enterocytes)							
	<i>CYP2C9</i>	<i>CYP2C19</i>	<i>CYP3A4M</i>	<i>CYP3A4T</i>	<i>CYP2J2</i>	<i>CES2</i>	<i>UGT</i>	<i>SULT</i>
HE3005	1.68	0.56	2.67	ND	1.2	0.23	8.38	8.72
HE3006	0.59	0.29	0.13	6.23	0.73	0.33	2.3	2.04
HE3007	0.91	0.39	0.99	22.7	0.57	0.46	3.08	4.04
HE3008	0.46	0.68	0.87	NA	0.33	0.55	1.8	1.79
HE3009	1.18	0.35	0.72	32.1	0.25	0.6	4.32	7.78
HE3010	1.21	0.62	0.46	17.5	0.99	0.41	2.56	3.32
HE3011	0.03	0.01	0.09	2.6	0.33	0.29	1.01	1.7
HE3014	0.44	0.11	0.4	8.2	1.18	0.17	3.55	2.66
HE3015	2.5	0.49	2.55	ND	0.95	0.51	7.33	5.23
HE3016	2.05	1.08	0.99	26.7	0.93	0.34	5.71	4.13
HE3019	0.24	0.11	0.3	5.27	0.26	0.3	1.47	1.89
HE3020	0.31	0.14	0.49	12.9	0.58	0.59	5.83	1.84
HE3021	0.2	0.06	0.17	2.97	0.19	0.08	1.49	1.64
HE3027	2.02	0.31	0.68	25.9	0.76	0.19	3.68	2.69
HE3028	0.68	0.21	0.82	26	0.71	0.3	3.84	9.02
HE3029	0.86	0.12	0.59	12.1	0.76	0.08	6.55	3.65
HE3030	0.26	0.28	0.29	14.9	0.32	0.05	15	8.3
HE3031	0.34	0.09	0.16	2.95	0.49	0.18	1.6	0.79
HE3032	1.84	0.81	1.3	43.4	0.82	0.24	5.9	4.5
HE3033	2.11	1.13	1.03	28.4	0.7	0.11	29.6	14.2
HE3034	7.93	0.71	4.35	45.2	1.98	0.12	122.6	8.24
HE3040	0.96	0.78	1.00	32.30	0.30	0.18	23.80	22.00
HE3042	0.23	0.03	1.66	31.20	1.06	0.06	74.20	8.98
HE3043	1.39	0.21	0.62	18.80	0.55	0.23	9.13	9.00
<i>mean</i>	<u>1.27</u>	<u>0.40</u>	<u>0.97</u>	<u>19.92</u>	<u>0.71</u>	<u>0.28</u>	<u>14.36</u>	<u>5.76</u>
<i>sd</i>	<u>1.59</u>	<u>0.33</u>	<u>0.99</u>	<u>12.99</u>	<u>0.41</u>	<u>0.17</u>	<u>27.74</u>	<u>4.88</u>

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 19, 2024

Cryopreserved human enterocytes

