Hepatic Versus Enteric Uridine 5'-diphospho (UDP)-glucuronosyltransferase (UGT) Activity: A Comparison Of Cryopreserved Human Hepatocytes (CHH) and Cryopreserved Intestinal Mucosa (CHIM) In The Enzyme Kinetics Of β-Estradiol, Chenodeoxycholic Acid, Propofol, Raloxifene, Serotonin, Trifluoperazine And Zidovudine Glucuronidation

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INTRODUCTION

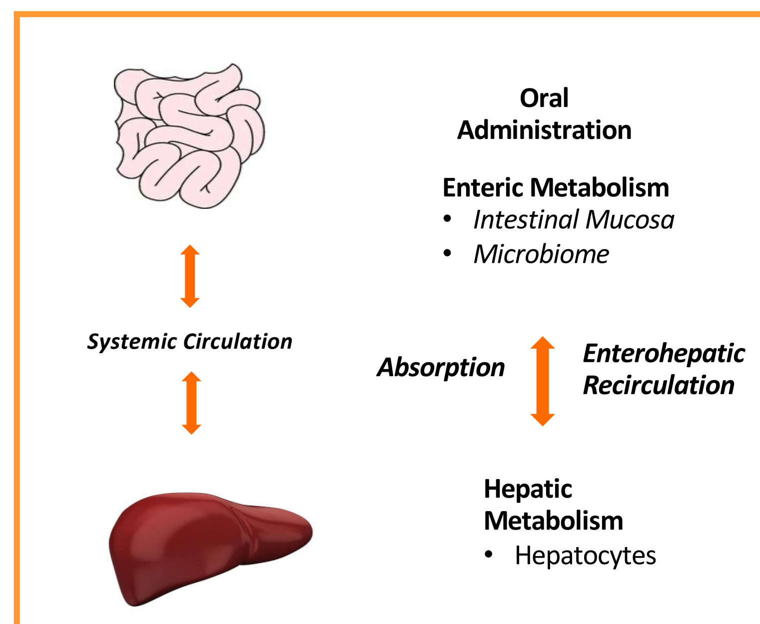
- An orally-administered drug is firstly metabolized enterically, either by the intestinal flora or by the enterocytes during the absorption process. Upon entering the portal circulation, the absorbed drug as well as enteric metabolites are further subjected to hepatic metabolism, followed by the distribution of the unmetabolized parent drug and the hepatic metabolites into the systemic circulation.
- An accurate assessment of the potential in vivo drug metabolic fates of orally administered drugs therefore would require a thorough understanding of both enteric and hepatic drug metabolism enzymes.
- Besides P450 isoforms, UGT activities are key to the pharmacokinetics, metabolic fate, drug-drug interactions, and drug toxicity
- We report here a comparison of human enteric and hepatic UGT isoform activities using cryopreserved human hepatocytes and cryopreserved human intestinal mucosa.

MATERIALS & METHODS

CHH and CHIM. *Cryopreserved* pooled donor human hepatocytes (PHH9001) and pooled donor intestinal mucosa (CHIM6031) from Discovery Life Sciences were used in the study. For experimentation, the cryopreserved vials were removed from liquid nitrogen storage and thawed in a 37°C water bath for approximately 2 min. The contents of each individual vial were decanted into a 50 ml conical tube containing Cryopreserved Hepatocyte or Enterocyte Recovery Medium, (UCRM or CERM, Discovery Life Sciences-IVAL, Columbia, MD) that was pre-warmed in a 37°C water bath. The thawed PHH or CHIM were recovered by centrifugation at 100 x g for 10 min at room temperature. After centrifugation, the supernatant was removed by decanting. Hepatocyte/Enterocyte Incubation Medium, (HQM™, In Vitro ADMET Laboratories, Columbia, MD) was added to the intact pellet of hepatocytes or enterocytes to constitute the cell suspensions for experimentation.

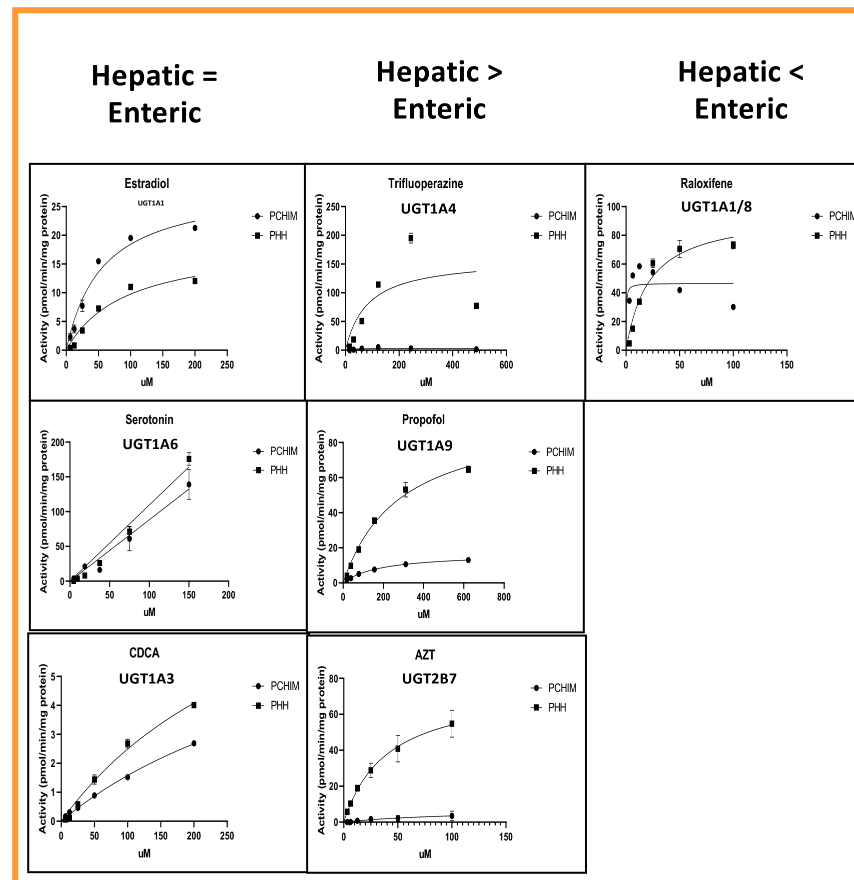
Quantification of UGT activities. The substrates and the metabolites quantified for the multiple UGT pathways evaluated are shown in Table 1. Incubations of CHIM and CHH with the various substrates were performed in a cell culture incubator maintained at 37°C with a humidified atmosphere of 5% CO_2. A volume of 50 mL of drug metabolizing enzyme substrates at 2x of the final desired concentrations was added into the designated wells of a 96 well plate (reaction plate) containing CHH and CHIM. The reaction plate was placed in a cell culture incubator for 15 minutes to prewarm the substrate solutions to 37°C, followed by addition of PHH/CHIM at a volume of 50 mL per well to initiate the reaction. The reaction plates were then incubated at 37°C for 30 minutes. All incubations were performed in triplicate. Metabolism was terminated in each well by the addition of 200 µl acetonitrile containing 250 nM of the internal standard tolbutamide. The incubated samples were stored at -80°C for the subsequent LC/MS-MS analysis.

An orally administered drug is subjected to sequential enteric and hepatic metabolism



UGT ISOFORM	SUBSTRATE	MARKER METABOLITE
UGT1A1	Beta-Estradiol	b-Estradiol 3-Glucuronide
UGT1A3	Chenodeoxycholic acid	CDCA Glucuronide
UGT1A9	Propofol	Propofol b-D-Glucuronide
UGT1A4	Trifluoperazine	Trifluoperazine Glucuronide
UGT1A1/8	Raloxifene	Raloxifene 6-Glucuronide
UGT1A6	Serotonin	Serotonin b-D-Glucuronide
UGT2B7	AZT	AZT Glucuronide

RESULTS: A Comparison of Hepatic and Enteric UGT Activities



Substrate	DME	PCHIM			PHH		
		Vmax	Km	CL	Vmax	Km	CL
AZT	UGT2B7	7.46	113.60	0.07	75.83	40.19	1.89
CDCA	UGT1A3	8.76	455.00	0.02	11.27	352.80	0.03
Estradiol	UGT1A1	28.74	55.96	0.51	18.78	92.47	0.20
Propofol	UGT1A9	16.95	187.00	0.09	96.71	285.20	0.34
Raloxifene	UGT1A1/8	46.61	0.30	155.89	95.78	21.62	4.43
Serotonin	UGT1A6	NA	NA	NA	NA	NA	NA
Trifluoperazine	UGT1A4	3.49	29.08	0.12	159.90	82.61	1.94

SUMMARY AND CONCLUSIONS

UGT activities were quantified in two in vitro experimental models, cryopreserved human hepatocytes and cryopreserved human intestinal mucosa using isoform-selective substrates. The results show robust UGT isoform-selective substrate metabolism in both in vitro systems. The comparison of hepatic and enteric UGT activities are as follows:

- Hepatic = Enteric: UGT1A1, UGT1A3 and UGT1A6
- Hepatic > Enteric: UGT1A4, UGT1A9 and UGT2B7
- Enteric > Hepatic: UGT1A8

Our results suggest that it is important to define both enteric and hepatic drug metabolism for a thorough understanding of the metabolic fate of an orally administered drug.

- Cryopreserved Human Hepatocytes and Cryopreserved Human Intestinal Mucosa represent in vitro experimental systems that can be used routinely for the definition of human-specific drug metabolism, including that mediated by hepatic and enteric UGT.

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