Isolation and Cryopreservation of Enterocytes for Metabolism and DDI Studies

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Introduction

Why Enterocytes:

- Key cell type for oral bioavailability
- First pass metabolism before the liver
- Intestinal DDI with orally co-administered substances (foods, nutrient supplements, drugs) Intestinal DDI may not occur in the liver due to lower hepatic exposure (e.g. grapefruit juice)

Experimental Objectives:

- Development of procedures for isolation and cryopreservation of human and animal enterocytes
- Characterization of cryopreserved enterocytes for ADME properties
- Application in the evaluation of intestinal DDI

<u>As of this writing, application of cryopreserved enterocytes for intestinal ADME evaluation, especially for intestinal drug metabolism, has not been reported.</u>

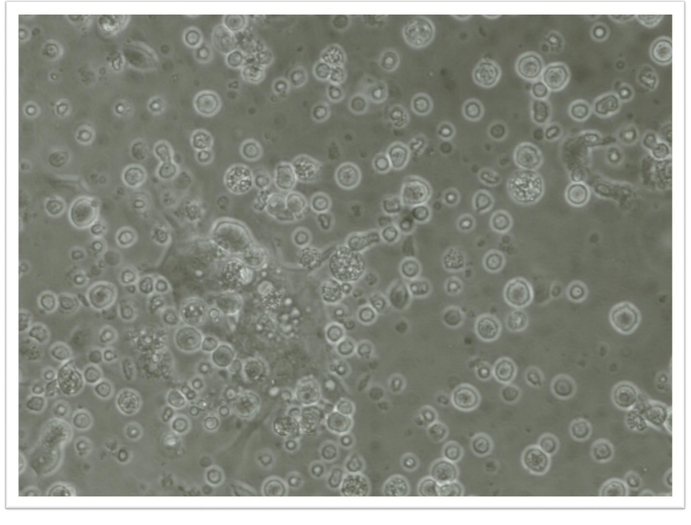
Materials & Methods

Enterocyte isolation and cryopreservation: Mouse enterocytes were isolated from CD-1 male mice. Human intestines were provided to us by IIAM from organ donors. The intestines (duodenum and proximal jejunum) were extensively washed with an isotonic salt solution to remove food residues and other contents. After washing, the intestine lumens were subjected to collagenase digestion at 37° C. The isolated enterocytes were purified by density centrifugation. They enterocytes were then cryopreserved and stored in liquid nitrogen.

P450 activity: CYP3A activity of the thawed enterocytes was evaluated using luciferin-IPA as substrate.

ADME gene expression: CYP and transporter enzymes were measured by RT-PCR.

Drug-drug Interaction: CYP3A4 activity of human enterocytes was measured in the presence of fruit juice (apple, orange, and grapefruit juice) in protein-free culture medium (HQM, IVAL), with pH adjusted to 7.25 using luciferin-IPA substrate with an incubation duration of 30 min.



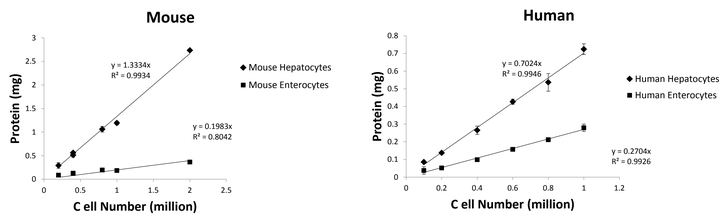
Morphology of thawed human enterocytes (Lot number HE3005)

The post-thaw viability (average of 5 independent thaws) was 89.42% based on trypan blue with a yield of 2.8 million cells per vial.

Results

Comparison of Enterocytes and Hepatocytes

Hepatocyte > enterocytes in cellular protein contents



Protein contents (mg protein/million cells):
Mouse: hepatocytes: 1.33, enterocytes: 0.20
Human: hepatocytes: 0.70, enterocytes: 0.27

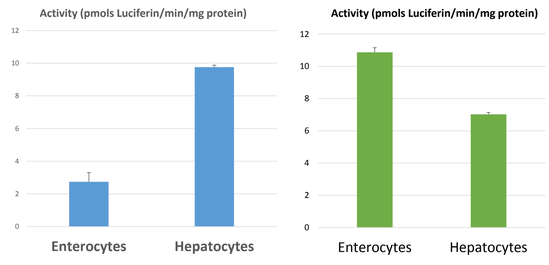
ADME Gene Expression: Human Enterocyte/Hepatocyte Ratio (E/H Ratio)

Gene	E/H ratio	Gene	E/H ratio
CYP1A2	0.000	*SLC10A1*	0.000
CYP2B6	0.002	*SLC22A1*	0.001
CYP3A4	**0.377**	*SLC22A7*	0.001
CYP2C8	0.001	*SLCO1B1*	0.000
CYP2C9	0.133	*SLCO1B3*	0.000
CYP2C19	0.730	*SLCO2B1*	**0.275**
CYP2D6	0.065	*ABCB1*	2.089
CYP3A5	0.120	*ABCB11*	0.000
AHR	1.265	*ABCC2*	**0.439**
CAR	0.020	*ABCC3*	0.669
PXR	**0.437**	*ABCC4*	5.615
ALB	0.000	*ABCG2*	8.624

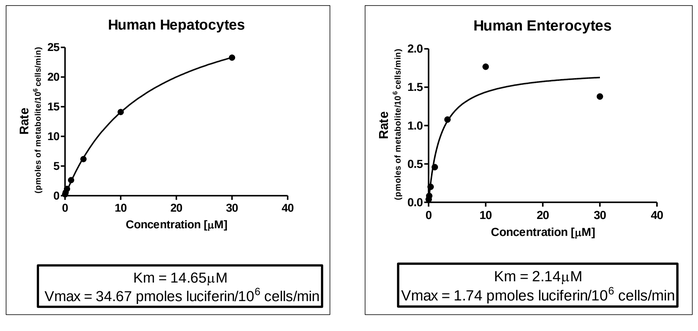
- *Enterocytes < Hepatocytes (E/H < 0.5, green background): CYPs (except 2C9) CAR, PXR, ALB, SLCs, ABCB11, and ABCC2, with CYP3A4, PXR, SLCO2B1, and ABCC2 having the highest ratios in this category. Enterocytes > Hepatocytes (E/H > 1.5, orange background): ABCB1, ABCC4, and ABCG2*

CYP3A4 Activity

Mouse



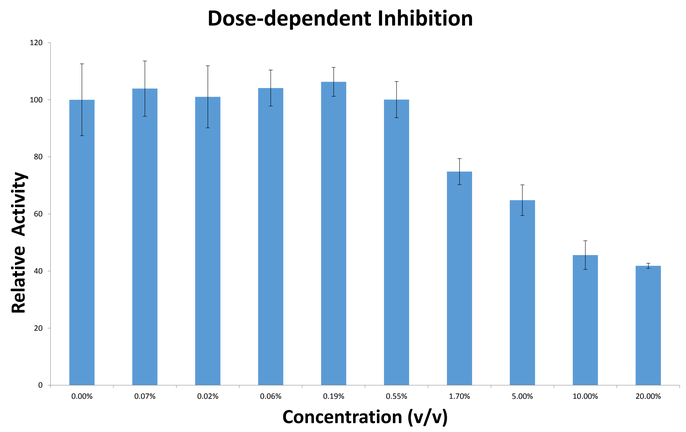
Human

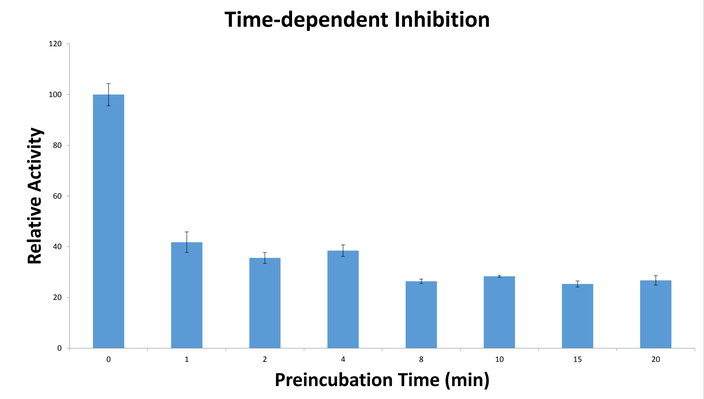


Higher affinity (lower Km) and lower capacity (lower Vmax) for enterocytes

Intestinal DDI

Grapefruit juice inhibition of enterocyte CYP3A4 activity





Conclusions

- Enterocytes can be effectively isolated and cryopreserved
- Gene expression of P450 isoforms and transporters in enterocytes are different than those in hepatocytes
- CYP3A4 activity is comparable to human hepatocytes
- Cryopreserved enterocytes can be effectively used to investigate clinically-observed time dependent inhibition of CYP3A4 by grapefruit juice

<u>Results suggest that cryopreserved enterocytes are useful for in vitro evaluation of intestinal metabolism, DDI, and toxicity assessments.</u>

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