# Human Enterocytes: Isolation, Cryopreservation, Characterization, and Application in The Evaluation of Drug-Food Interactions

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## Introduction

### *Why Enterocytes*

- **Key cell type for oral bioavailability (as a function of permeability, metabolism, efflux)**
- **First pass metabolism before the liver**
- **Target of drug-interactions with orally co-administered substances**
- **Target of enterotoxicity of ingested toxicants**
- **Target of inflammatory bowel disease**

## Materials & Methods

- ***Enterocyte isolation and cryopreservation:*** *Enterocytes were isolated and purified from human duodenum segments obtained from the International Institute for the Advancement of Medicine (IIAM, Exton, PA) using a proprietary method. The cryopreserved enterocytes were stored in the vapor phase of liquid nitrogen storage containers.*
- ***Recovery of cryopreserved enterocytes:*** *Cryopreserved enterocytes (In Vitro ADMET Laboratories, Columbia, MD) were thawed in a 37°C water bath and recovered in Cryopreserved Enterocyte Recovery Medium, (CERM™, In Vitro ADMET Laboratories, Columbia, MD). Viability and yield were quantified in a hemacytometer based on dye exclusion (Trypan Blue; Sigma-Aldrich, St. Louis, MO).*
- ***Cryopreserved hepatocytes:*** *Gene expression of cryopreserved enterocytes was compared to cryopreserved hepatocytes using human hepatocytes pooled from 30 donors (PHH8006; In Vitro ADMET Laboratories, Columbia, MD).*
- ***Quantification of gene expression:*** *Total RNA was isolated individually from each lot of hepatocytes and enterocytes by RNeasy kit (Qiagen, Valencia, CA), cDNA was synthesized from each sample by use of High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). Real-time reactions were carried out using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems). Each PCR cycle threshold (Ct) was normalized to the average Ct of the endogenous housekeeping control gene GAPDH. The comparative Δ Ct method was used to calculate relative quantification of gene expression.*
- ***Incubation of enterocytes with drug metabolizing enzyme substrates:*** *Drug metabolizing enzyme substrate incubations were performed in 96-well plates with 150,000 enterocytes/well. Final concentrations of the P450 substrates were: 25 µM of diclofenac (CYP2C9), 250 µM of s-mephenytoin (CYP2C19), 20 µM of midazolam (CYP3A4/5), 100 µM of 7-hydroxycoumarin (UGT, SULT), 50 µM of astemizole (CYP2J2), and 50 µM of irinotecan (CES2). Metabolism was terminated in each well by the addition of 100 µl acetonitrile containing internal standards of either 10 nM terfenadine or 50 nM tolbutamide. LC/MS-MS quantitation of metabolite formation was performed using an API 4000 QTRAP mass spectrometer with an electrospray ionization source (AB SCIEX, Framingham, MA) connected to Agilent 1200 series high performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA).*
- ***Fruit juice-drug interaction evaluation:*** *Effects of fruit juice on enterocyte CYP3A4 activity were evaluated using luciferin IPA (LIPA ; Promega, Madison, WI) with luminescence quantified on a Perkin Elmer Wallac 1420 Victor microplate reader. Fruit juices were diluted with HQM (IVAL, Columbia, MD) with pH adjusted to 7.0.*

## Results

### *Successful Isolation and Cryopreservation from Multiple Donors*

| Lot | Gender | Race | Age (Years) |
|---|---|---|---|
| HE3005 | M | C | 23 |
| HE3006 | M | C | 47 |
| HE3007 | F | C | 44 |
| HE3008 | M | C | 18 |
| HE3009 | F | C | 44 |
| HE3010 | F | C | 50 |
| HE3011 | M | H | 43 |
| HE3013 | F | AA | 57 |
| HE3014 | M | AA | 49 |
| HE3015 | M | C | 24 |
| HE3029 | M | AA | 32 |
| HE3027 | M | C | 25 |

### *Morphology*

### *Biomarker Gene Expression*

***(*Hepatocyte Biomarker; **Enterocyte Biomarker)***

| Gene name | $2^{-\Delta Ct}$ Enterocytes (HE3005) | $2^{-\Delta Ct}$ Hepatocytes (PHH8006) | Ratio of enterocyte to hepatocytes |
|---|---|---|---|
| *ALB (Albumin)** | ND | 34.9 | NA |
| *SI (Sucrose Isomaltase)*** | 48.6 | ND | NA |
| *MAGM (Maltase Glucoamylase )*** | 40.7 | ND | NA |

### *Comparison of Enterocytes and Hepatocytes in Gene Expression*

*P450 Isoforms*

| Gene name | $2^{-\Delta Ct}$ enterocytes | $2^{-\Delta Ct}$ hepatocytes | Ratio of enterocyte to hepatocytes |
|---|---|---|---|
| **CYP1A2** | 0.0000 | 0.1333 | 0.00 |
| **CYP2B6** | 0.0024 | 1.0304 | 0.00 |
| **CYP3A4** | 0.5989 | 1.5889 | 0.38 |
| **CYP2C8** | 0.0032 | 2.6429 | 0.00 |
| **CYP2C9** | 0.2267 | 1.7006 | 0.13 |
| **CYP2C19** | 0.1259 | 0.1726 | 0.73 |
| **CYP2D6** | 0.0004 | 0.0057 | 0.07 |
| **CYP3A5** | 0.0508 | 0.4226 | 0.12 |
| **CYP2J2** | 1.1584 | 0.8087 | 1.43 |
| **CYP2S1** | 0.2513 | 0.0027 | **93.51** |
| **CYP4F12** | 0.5504 | 0.5211 | 1.06 |
| **CYP1A1** | 0.0002 | 0.0131 | 0.01 |

*Phase II Drug Metabolizing Enzymes*

| Gene name | $2^{-\Delta Ct}$ enterocytes | $2^{-\Delta Ct}$ hepatocytes | Ratio of enterocyte to hepatocytes |
|---|---|---|---|
| **UGT1A1** | 1.6996 | 1.6743 | 1.02 |
| **SULT1A1** | 0.5677 | 0.7652 | 0.74 |
| **GSTP1** | 0.9673 | 0.0591 | 16.36 |
| **CES1** | 0.0017 | 2.3341 | 0.00 |
| **CES2** | 10.8464 | 6.2252 | 1.74 |

*Uptake and Efflux Transporters*

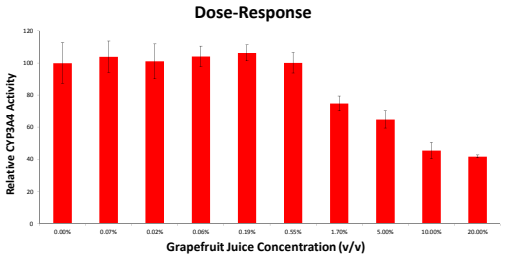
| Transporter | $2^{-\Delta Ct}$enterocytes | $2^{-\Delta Ct}$ hepatocytes | Ratio of enterocyte to hepatocytes |
|---|---|---|---|
| **NTCP** | ND | 0.2677 | NA |
| **OCT1** | 0.0007 | 0.6346 | 0.0011 |
| **OAT2** | 0.0000 | 0.0148 | 0.0009 |
| **OATP1B1** | 0.0000 | 0.1206 | 0.0001 |
| **OATP1B3** | 0.0000 | 0.3432 | 0.0000 |
| **OATP2B1** | 0.0798 | 0.2900 | 0.2752 |
| **OATP1A2** | 0.0000 | 0.3333 | 0.0000 |
| **PEPT1** | 0.4371 | 0.0294 | 14.8909 |
| **OCTN1** | 0.0011 | 0.0006 | 1.7188 |
| **OCTN2** | 0.0443 | 0.0076 | 5.8240 |
| **MDR1** | 0.1996 | 0.0955 | 2.0892 |
| **BSEP** | 0.0000 | 0.1635 | 0.0001 |
| **MRP2** | 0.1632 | 0.3714 | 0.4394 |
| **MRP3** | 0.0423 | 0.0631 | 0.6693 |
| **MRP4** | 0.0182 | 0.0032 | 5.6154 |
| **BCRP** | 0.0949 | 0.0110 | 8.6239 |

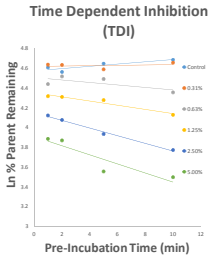
Human hepatocytes Lot: PHH8006 (pool hepatocytes); Human enterocytes Lot: HE3005.

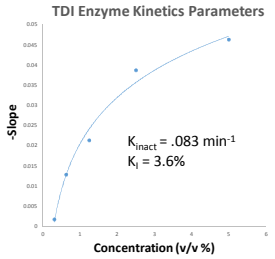
### *Drug Metabolizing Enzyme Activities* *(pmol/min/million enterocytes)*

| *Drug Metabolizing Enzyme* | *Substrate* | *Substrate Conc. (µM)* | *Metabolite* | *HE3005* | *HE3006* | *HE3007* | *HE3008* | *HE3009* | *HE3010* | *HE3011* | *HE3014* | *HE3015* | *HE3016* | *HE3020* | *HE3027* | *HE3029* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CYP2C9 | Diclofenac | 25 | 4-OH Diclofenac | 1.68 | 0.59 | 0.91 | 0.46 | 1.18 | 1.21 | 0.03 | 0.44 | 2.50 | 2.05 | 0.31 | 2.02 | 0.86 |
| CYP3A4/5 | Midazolam | 20 | 1-OH-Midazolam | 2.67 | 0.13 | 0.99 | 0.87 | 0.72 | 0.46 | 0.09 | 0.40 | 2.55 | 0.99 | 0.49 | 0.68 | 0.59 |
| UGT | 7-OH Coumarin | 100 | 7-OH Coumarin Glucuronide | 8.38 | 2.30 | 3.08 | 1.80 | 4.32 | 2.56 | 1.01 | 3.55 | 7.33 | 5.71 | 5.83 | 3.68 | 6.55 |
| Sulfate Transferase | 7-OH Coumarin | 100 | 7-OH Coumarin Sulfate | 8.72 | 2.04 | 4.04 | 1.79 | 7.78 | 3.32 | 1.70 | 2.66 | 5.23 | 4.13 | 1.84 | 2.69 | 3.65 |
| CYP2J2 | Astemizole | 50 | O-Demethyl Astemizole | 1.20 | 0.73 | 0.57 | 0.33 | 0.25 | 0.99 | 0.33 | 1.18 | 0.95 | 0.93 | 0.58 | 0.76 | 0.76 |
| CES2 | Irinotecan | 50 | SN38 | 0.23 | 0.33 | 0.46 | 0.55 | 0.60 | 0.41 | 0.29 | 0.17 | 0.51 | 0.34 | 0.59 | 0.19 | 0.08 |

### *Grapefruit Juice Inhibition of Enterocyte CYP3A4 Activity*




## Summary

- **Primary isolates of human enterocytes were successfully cryopreserved to retain drug metabolizing enzyme activities**
- **The identity of the enterocytes was confirmed by their expression of the biomarker genes, SI and MAGM**
- **Significant differences were observed between hepatocytes and enterocytes in the gene expression of drug metabolizing enzymes, uptake and efflux transporters**
- **The cryopreserved enterocytes were active in both Phase I oxidative and Phase II conjugative drug metabolism**
- **The known grapefruit juice inhibition of CYP3A4 activity was reproduced in the cryopreserved human enterocytes**

## Conclusions

- ***We are the first to report successful isolation and cryopreservation of human enterocytes.***
- ***The enterocytes were cryopreserved after isolation without culturing, thereby allowing the retention of drug metabolizing enzyme activities representative of the intestines in vivo***
- ***The cryopreserved enterocytes represent a physiologically relevant and convenient experimental model for the evaluation of intestinal drug metabolism, drug-drug/food-drug interactions, and enterotoxicity***

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