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 toxins
- Target of inflammatory bowel disease

Materials & Methods

- Successful isolation and cryopreservation of enterocytes were successfully cryopreserved to retain drug metabolizing enzyme activities
- 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
- Enterocytes were cryopreserved after isolation without culturing, thereby allowing the retention of drug metabolizing enzyme activities representative of the intestines in vivo
- 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, 150 µM of tolbutamide, and 100 µM of verapamil. 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 CYP3A4 reporter gene constructs transfected into HEK293T cells, and the activity was measured using luciferin (LIPAM; Promega, Madison, WI) with luminescence quantified on a Perkin Elmer Wallac 1420 Victor microplate reader. Fruit juices were diluted 1:20 with HQM (IVAL, Columbia, MD) with pH adjusted to 7.0

Results

Comparison of Enterocytes and Hepatocytes in Gene Expression

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<th>Enterocytes</th>
<th>Hepatocytes</th>
<th>Ratio of enterocyte to hepatocyte</th>
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<tr>
<td>2\textsuperscript{nd} enterocytes</td>
<td>2\textsuperscript{nd} hepatocytes</td>
<td>Ratio of enterocyte to hepatocyte</td>
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Grapefruit Juice Inhibition of Enterocyte CYP3A4 Activity

- Dose-Response

Uptake and Efflux Transporters

Biomarker Gene Expression

(*Hepatocyte Biomarker; **Enterocyte Biomarker)

- Oral uptake was measured using luciferin (LIPAM; Promega, Madison, WI) with luminescence quantified on a Perkin Elmer Wallac 1420 Victor microplate reader. Fruit juices were diluted 1:20 with HQM (IVAL, Columbia, MD) with pH adjusted to 7.0

Materials & Methods

- Enterocyte and hepatocyte cultures were incubated for 24 hours prior to performing the TDI test to measure the activities of the P450 enzymes involved in drug metabolism

Significance

- The known grapefruit juice inhibition of CYP3A4 activity was reproduced in the cryopreserved human enterocytes

Summary

- 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 oxidant 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