ENTERIC VERSUS HEPATIC METABOLISM: A COMPARISON CRYOPRESERVED HUMAN INTESTINAL MUCOSA (PCHIM) AND CRYOPRESERVED HUMAN HEPATOCYTES (PHH) IN DRUG METABOLIZING ENZYME ACTIVITIES

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A Need for an In Vitro Human Enteric System

- Oral administration is the preferred route of drug administration
- Enteric metabolism is known to be a major determinant of the bioavailability of orally administered drugs
- We have previously developed cryopreserved enterocytes for the evaluation of enteric drug metabolism (Ho et al., 2017, DMD), and most recently, cryopreserved human intestinal mucosa (CHIM; Li et al., 2016, DMD).
- An orally-administered drug travels within the intestinal tract and thereby is subjected to metabolism by the intestinal mucosal epithelium and the various regions.
- It is therefore important to define the drug metabolizing enzyme activities at various regions of the small intestine.
- We report here the drug metabolizing enzyme activities in CHIM from 10 consecutive 12-inch segments of the human small intestines.

Experimental Procedures

Hepatic Intestine, Human small intestines were obtained from the International Institute for the Investigation of Cancer, strap frozen, and stored at −80 °C. Enteric mucosal epithelial tissue was obtained from resected segments of the intestinal tissues from the intestinal tract. The intestinal tissue fragments were collected in cold physiological saline and stored at −80 °C. Enteric fragments were incubated in cold Ringer’s solution for 15 min, followed by homogenization using a programmable liquid nitrogen cell homogenizer. The homogenate was then filtered through a 70 µm nylon mesh filter to remove tissue debris. Enteric tissue was used within 2 min of collection.

Preparation of CHIM from Human Small Intestine

1. Dissect into 10 consecutive 12-inch segments, starting right after the pyloric valve
2. Collagenase Digestion
3. Gentle Homogenization of the Isolated Intestinal Villi
4. Cryopreservation

RESULTS

Hepatic (Pooled Human Hepatocytes, PHH) vs Enteric (Pooled CHIM, PCHIM) in Drug Metabolism Enzyme Activities

Enzyme Kinetics: Enteric vs Hepatic CYP3A4 and UGT

<table>
<thead>
<tr>
<th>Substrate</th>
<th>DME</th>
<th>PCHIM</th>
<th>PHH</th>
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<td>7.46</td>
<td>113.60</td>
<td>82.61</td>
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<tr>
<td>Serotonin</td>
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<td>Trifluoperazine</td>
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<tr>
<td>Testosterone</td>
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<td>39.48</td>
<td>113.60</td>
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</table>

Summary

The major observations are as follows:

1. Overall DME activities:
   - Enteric = Hepatic (CHIM-L2A-PHIM) vs (PHH-L2C)
   - Enteric = Hepatic (CHIM-L2A-PHIM) vs (PHH-L2C)
   - Enteric = Hepatic (CHIM-L2A-PHIM) vs (PHH-L2C)
   - Enteric = Hepatic (CHIM-L2A-PHIM) vs (PHH-L2C)

2. CYP3A4 enzyme kinetics: Enteric vs Hepatic in Km, Vmax, and Cl for both midazolam and testosterone

3. UGT-mediated metabolic clearance:
   - Enteric = Hepatic (CHIM-L2A-PHIM) vs (PHH-L2C)
   - Enteric = Hepatic (CHIM-L2A-PHIM) vs (PHH-L2C)
   - Enteric = Hepatic (CHIM-L2A-PHIM) vs (PHH-L2C)

References