Enzyme Activity and Transporter Facilitated Uptake Comparisons: Multi-donor Pooled Cryopreserved Hepatocytes (HEP10™) to Single Donor Constituent Lots

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ABSTRACT/INTRODUCTION

Primary hepatocytes represent an important research tool due to their ability to effectively model the human liver in response to drug and chemical exposure and are accepted by the FDA for in vitro ADME/Tox metabolism studies (Nagilla et al., 2006; USFDA, 2006). Cryopreserved hepatocytes offer convenience, consistency, and retain the ability to model the human liver in response to xenobiotic exposure (Smith et al., 2012). However, individual donor variability can result in widely ranging, idiosyncratic results during in vitro studies (Kaplowitz, 2005). In this report, we demonstrate that pooling multiple donors can produce a more comprehensive representation of the population. Therefore, multi-donor pooled hepatocytes are ideal for xenobiotic metabolism, clearance, screening, transporter uptake, and drug-drug interaction studies. We propose that multi-donor pooled hepatocytes could promote consistency in the single donor metabolic capacity, while minimizing variability, resulting in a more robust in vitro model for ADME/Tox.

MATERIALS AND METHODS

Cryopreserved hepatocyte recovery media [CHRM™–CM7000 (GIBCO®), plating media [William’s E Medium (WEM)], plating supplement pack [serum-containing, CM3000 [GIBCO®], and incubation media [WEM, no Phenol Red, containing 15mM HEPES]) were obtained from Life Technologies (Carlsbad, CA). Additional chemicals were purchased from Sigma Aldrich and Fisher Scientific. Whole liver tissues and resected liver tissues were procured from rejected-for-transplant livers or participating medical centers. Hepatocytes were isolated using a modified two-step collagenase digestion procedure and immediately cryopreserved as single donors (LeCluyse et al., 2005). HEP10™ pooled hepatocytes were produced using CellStream Isolation Technology™.

Cell Health Assessment:

Multi-donor pooled cryopreserved hepatocytes, HEP10™, and single donor cryopreserved hepatocytes were thawed in a 37°C water bath and transferred into CHRM™ and centrifuged at 100g for 10 minutes. Supernatants were removed and cells were resuspended in approximately 1 mL of plating media (WEM and CM3000) per million total cells and analyzed for viability via Trypan Blue exclusion. Viabilities of cryopreserved HEP10™ pooled hepatocytes and individual donors met the acceptance criteria of >75%. Cell morphology assessments were determined by observation using an inverted research microscope equipped with phase-contrast optics, a 3 CCD camera, and imaging computer with image analysis software. Morphology was visually inspected for markers of cellular stress including: nucleus integrity/size/shape, cytosolic clarity, cell membrane integrity, organelle size, lipid droplets/content, cell debris, and cell excretion products. Viability determination via Trypan Blue exclusion was repeated after a 2-hour stability in suspension at 37°C.

Metabolic Activity Assessment:

Functional metabolic assessments of Phase I enzymes were performed on primary hepatocytes in suspension using probe substrates phenacetin (CYP1A2), bupropion (CYP2B6), paclitaxel (CYP2C8), diclofenac (CYP2C9), S-mephenytoin (CYP2C19), dextromethorphan (CYP2D6), chlorzoxazone (CYP2E1), testosterone (CYP3A4/5), midazolam (CYP3A4/5) and benzydamine (FMO). Phase II activities to probe for glucuronidation (UGT) and sulfation (7HCS) were assessed by analysis of radiolabeled metabolites. Radiolabeled substrates taurocholate, 1 µM, and estradiol 17-β-glucuronide, 1 µM, at 37°C and 80⁰C prior to analysis. LC-MS/MS methods were used for analysis of radiolabeled metabolites. Data for prediction of in vivo clearance. J Pharmacol Toxicol Methods 53(2):106-116.

RESULTS

Table 3: Comparison of Active Transporter Uptake (pmol/min/mg protein)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Donor Type</th>
<th>Min.</th>
<th>Median</th>
<th>Max.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>N</th>
<th>Levene (p-value)</th>
<th>Goodness-of-Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurocholate</td>
<td>HEP10™</td>
<td>6.2</td>
<td>27.7</td>
<td>84.7</td>
<td>43.0</td>
<td>23.1</td>
<td>13</td>
<td>0.2192</td>
<td>0.2805</td>
</tr>
<tr>
<td>Midazolam</td>
<td>HEP10™</td>
<td>1.0</td>
<td>11.1</td>
<td>28.8</td>
<td>11.4</td>
<td>7.6</td>
<td>28</td>
<td>0.0024*</td>
<td>0.0215*</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The data comparing HEP10™ multi-donor pooled hepatocytes against the individual donor lots demonstrate a lower variability when compared to the distribution of individual hepatocyte donors.

REFERENCES


USFDA. 2006. Draft guidance for industry: Drug interaction studies—Study design, data analysis, and implications for dosing and labeling. Silver Spring, Maryland: USFDA.


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