Primary human hepatocyte cultures are the gold standard for preclinical drug safety assessment as well as required by the FDA for evaluation of drug induction potential. Cryopreserved human hepatocytes have been validated as a comparable model to fresh human hepatocytes. However, these cultures are limited by their relatively short life-span of 5 to 7 days accompanied by a rapid drop in metabolic function which precludes, among other experiments, evaluation of low metabolic turnover compounds, multi-day toxicity studies, and long-term viral infection studies. To address this need, we are evaluating a new cell culture media that maintains or enhances the function and viability of cryopreserved human hepatocytes for at least 10 days. The new culture media has been tested for the ability to prolong hepatocyte life as determined by morphological assessment for polarity, bile canaliculi formation using carboxy dextrorhodamine diacetate (CDFDA) assay, and ATP content. HepExtend™ maintained cell viability for 10-14 days as evidenced by polarization of cells, presence of bile canaliculi, and sustained ATP levels. Hepatocytes cultured in the new long term culture optimized media displayed CYPIA2, CYPS6B, and CYPS4A4 activity levels significantly greater than those achieved using standard culture media after 5 days. Additionally, sulfotransferase and glucuronidation activities in cultures with HepExtend™ supplementation were significantly higher than standard culture controls after day 3 (SULT) and Day 7 (UGT), respectively. Cells maintained in the new media survived for at least 10 days (2-14 days in some cases) with metabolic activities comparable to day 5; this was not achievable with standard culture media. Our data indicates that optimizing the culture medium for primary cryopreserved human hepatocytes enables researchers to perform toxicity and metabolism assays that may more closely resemble clinical outcomes for pharmacological drugs.

**RESULTS**

**Figure 1.** Morphological assessment of 14 day cultures of cryopreserved primary human hepatocytes

Cryopreserved primary human hepatocytes were plated and cultured in standard William’s E Maintenance Media or William’s E Maintenance Media supplemented with HepExtend™ for 14 days. Cells were assessed for retention of markers of hepatocyte viability including: bright nuclei, bile canaliculi formation, cuboidal shape, integrity of monolayer, accumulation of dead cells.

**Figure 2.** Cytochrome P450 activity as a function of time

Culturing with HepExtend™ extends the viable culture life of primary human hepatocytes for 10-14 days compared to standard Maintenance Media. Cryopreserved primary human hepatocytes were plated and cultured in standard William’s E Maintenance Media or William’s E Maintenance Media supplemented with HepExtend™ for 14 days. Cells were assessed for retention of markers of hepatocyte viability including: bright nuclei, bile canaliculi formation, cuboidal shape, integrity of monolayer, accumulation of dead cells.

**Figure 3.** UGT and SULT activity as a function of time

Glucuronidation and sulfotransferase activities are rescued by culturing in HepExtend™ supplemented media. Cryopreserved primary human hepatocytes were plated and cultured in standard William’s E Maintenance Media (black line) or William’s E Maintenance Media supplemented with HepExtend™ (Blue line) for 14 days. Enzyme activities (UGT, SULT) were determined on indicated days.

**Figure 4.** Total ATP content of cryopreserved primary human hepatocytes as a function of time

Culturing with HepExtend™ extends the viable culture life of primary human hepatocytes for 10 days compared to standard Maintenance Media. Cryopreserved primary human hepatocytes were plated and cultured in standard William’s E Maintenance Media or William’s E Maintenance Media supplemented with HepExtend™ for 10 days. Total ATP content was determined using the Promega CellTiter-Glo® Luminescent Cell Viability Assay.

**CONCLUSIONS**

- Our HepExtend™ Supplement (50X) allows for long (≥10 days) culturing of primary human hepatocytes with maintenance of hepatocyte polarity, transport function, and basal enzymatic activities.
- Future studies will examine the effects of HepExtend™ supplementation on toxicity assays.
- For additional data on the effects of HepExtend™ supplementation on metabolism in primary human hepatocytes please visit Poster #14 "The Use of Cryopreserved Plateable Hepatocytes in Conjunction with HepExtend™ Media for Clearance Prediction of Metabolically Stable Compounds".

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