Optimization of Primary Hepatocyte Media for Evaluation of Drug Metabolism and Drug-Induced Liver Injury

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ABSTRACT

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 dichlorofluorescein 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 CYP1A2, CYP2B6, and CYP3A4 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 increased over standard culture controls after Day 3 (SULT) and Day 7 (UGT), respectively. Cultures maintained in the new media survived for at least 10 days (≥ 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 pharmaceutical drugs.

INTRODUCTION

Current standard monolayer human hepatocyte cultures are typically functional in culture for 5 to 7 days. While this is sufficient for induction studies and high rate of metabolic turnover compounds, there is an increasing need for longer term cultures of hepatocytes to study low turnover compounds as well as longer term toxicity studies. Recently developed coculture and 3D models have significantly increased the functional life span of hepatocytes in culture. However, many of these models rely on coculturing of human hepatocytes with other species of cells, are not amenable to high throughput processes that have been developed with standard culture practices, and are very costly. To address these concerns and extend the functional life of hepatocytes in culture, we have developed a new supplement, HepExtend™ Supplement (50X), that is used in conjunction with our standard William's E Maintenance media. To address the inherent variability of hepatocytes, HepExtend™ was developed using >13 different lots of plateable cryopreserved primary human hepatocytes across all three SKUs currently offered by Thermo Fisher Scientific (Metabolism, Induction, Transporter). Our data indicates that HepExtend™ enhances the viability and metabolic function of human hepatocytes for at least 10 days.

MATERIALS AND METHODS

Cell Culture Cryopreserved primary human hepatocytes (Thermo Fisher Scientific (TFS), HMCPIS, HMCPTS, HMCPMS) were briefly thawed at 37° , transferred to a 50 mL conical tube of Hepatocyte Thaw Media (TFS, CM7500), and centrifuged for 10 min at 100 x *g*. Cells were resuspended with William's E media (TFS, A1217601) supplemented with serum-containing Hepatocyte Thaw and Plating Media (TFS, CM3000) and plated at a density of 0.8 x10^6 cells/mL on 24-well collagen I coated plates (TFS, A11428-2). Media was replaced 4 hours after plating with William's E media supplemented with serum-free Hepatocyte Maintenance Supplements (i.e. William's E Maintenance Media; TFS, CM4000) or William's E Maintenance Media supplemented with HepExtend™ Supplement. An overlay of Geltrex® Basement Membrane Matrix (TFS, A1413201) diluted in William's E Maintenance media was applied at the 4 hour media change. Plating is Day 0. Media was changed daily for the duration of culture.

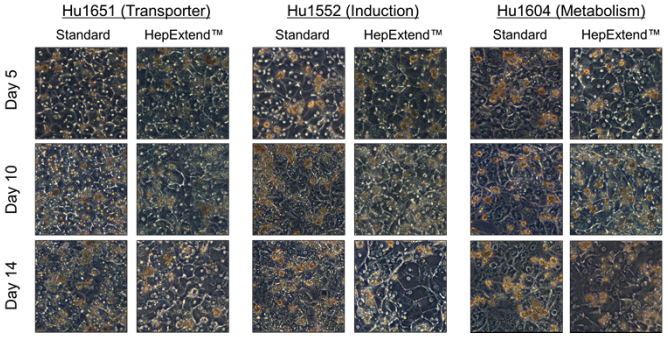
Enzymatic Activity On indicated days, cultures were washed with fresh William's E Maintenance Media and incubated with the following probe substrates for 15 minutes: CYP1A2-phenacetin; CYP2B6-bupropion; CYP2D6-dextromethorphan; CYP3A4-testosterone. For UGT and SULT activity determination, cells were incubated for 30 minutes with 7-hydroxycoumarin. After incubation, supernatants were collected and frozen until analysis. Metabolites were detected using LC/MS/MS or HPLC. Results are normalized to total protein content and incubation time.

CDFDA Assay Three lots of primary human hepatocytes were plated on collagen-I coated 24 well plates as described above. On indicated days, 5 μM CDFDA was prepared in standard Maintenance Media and exposed to cells for 20 min. Cells were washed 3x with media and images were captured using a fluorescence microscope.

ATP Analysis Five lots of primary human hepatocytes were plated on collagen-I coated opaque 96-well plates as described above. Geltrex overlay was applied 4 hours after plating. Total ATP content was assessed daily using the Promega CellTiter-Glo® Cell Viability Assay kit according to manufacturer instructions. An ATP standard curve was also run with each plate to quantitate ATP content.

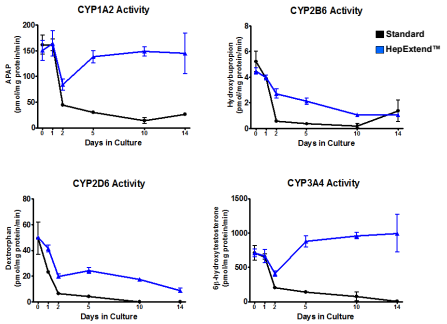
RESULTS

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



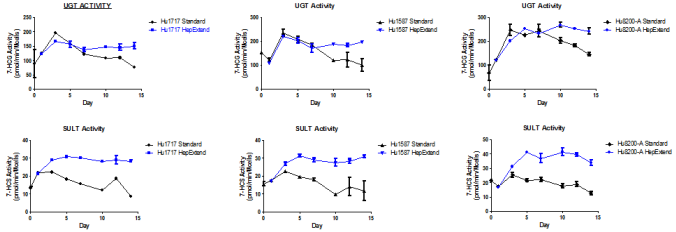
Culturing with HepExtend™ extends the viable culture life of primary human hepatocytes for 10-14 days compared to standard Maintenance Media. Cryopreserved primary human hepatocytes were plated and cultured in standard William's E Maintenance Media or William's E Maintenance Media supplemented with HepExtend™ 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 2. Cytochrome P450 activity as a function of time



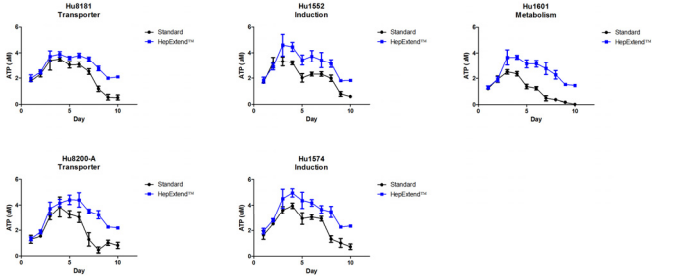
Cytochrome P450 activity is rescued by culturing in HepExtend™ supplemented media. Cryopreserved primary human hepatocytes (Hu1717) were plated and cultured in standard William's E Maintenance Media (black line) or William's E Maintenance Media supplemented with HepExtend™ (Blue line) for 14 days. Isoform-specific activities were determined on day 0 (suspension) and days 1, 2, 5, 10, and 14 as described in Materials and Methods.

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



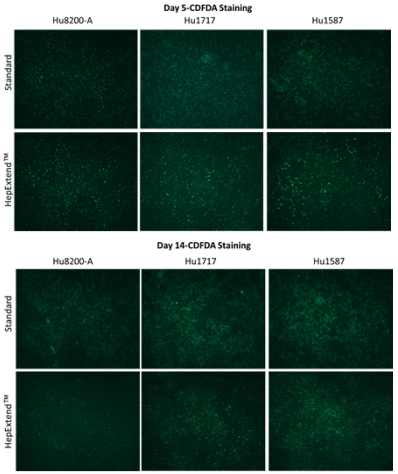
Glucuronidation and sulfotransferase activities are rescued by culturing in HepExtend™ supplemented media. Cryopreserved primary human hepatocytes were plated and cultured in standard William's E Maintenance Media (black line) or William's E Maintenance Media supplemented with HepExtend™ (Blue line) 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.

Figure 5. Bile canaliculi function as determined by CDFDA assay



HepExtend™ prolongs maintenance of bile canaliculi in 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. Fluorescent CDF is excreted in bile canaliculi, appearing as sharp dots and lines. The diffuse green background is caused by non-specific fluorescence of hepatocytes and incomplete excretion of CDF. The persistance of bile canaliculi staining suggests maintenance of transporter function in the presence of HepExtend™.

CONCLUSIONS

- Our HepExtend™ Supplement (50X) allows for long term (>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"**

ACKNOWLEDGEMENTS

The HepExtend™ Supplement (50X) was developed by the team of Jessica Bonzo, Kristen Vaverchak, Wendy Bray, Timothy Hurley, Chelsie Fritz, and Hanna Nguyen. HepExtend™ formulation assistance was additionally provided by Shyam Kumar and Lauren Sangenario, Cell Biology R&D.