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. The need for longer term hepatocyte cultures has increased with recent initiatives by European regulatory bodies to limit animal testing. To address this need, we are evaluating a new cell culture media supplement (HepExtend™) that maintains 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. In addition, to understand the effect of long term culture on metabolic function, Phase I enzymatic activities have been assessed by LC/MS/MS. Hepatocytes cultured in HepExtend™-supplemented media displayed CYP1A2, CYP2B6, CYP2D6, and CYP3A4 activity levels significantly greater than those achieved using standard culture media after 5 days. Cultures maintained in the new media survived for at least 10 days with metabolic activities comparable to day 5; this was not achievable with standard culture media. In addition supporting data shows hepatic cultures in the new media after 10 days maintained polarity and bile canalicul formation. As our data suggests, Hepextend™-supplemented media supports hepatocyte health and metabolic function for an extended period of time. This enables researchers to perform metabolic and toxicology experiments not achievable using currently available standard culture conditions. This further allows for multi-day toxicity studies and more predictive data interpretation.

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, iPS-, human umbilical cord, 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. This involves the inherent viability of hepatocytes was increased by utilizing Hepextend™, was developed using 13 different lots of plateable cryopreserved primary human hepatocytes across all three SKUs currently offered by Life Technologies (Metabolism, Induction, Transporter). Our data indicates that Hepextend™ enhances the viability and P450 function of human hepatocytes for at least 10 days.

MATERIALS AND METHODS

Cell Culture

Cryopreserved primary human hepatocytes (Life Technologies, HMPCIS, HMPCIT5, HMPC15) were briefly thawed at 37°C, transferred to a 50 ml conical tube of Hepatocyte Tissue Media (Life Technologies, CM1790), and centrifuged for 10 min at 100 x g. Cells were resuspended with William’s E media (Life Technologies, A1231401) supplemented with serum-free Hepatocyte Tissue and Plating Media (Life Technologies, CM3000) and plated at a density of 0.4 x 10^4 cells/mL on 24-well collagen I coated plates (Life Technologies, A14289-2). Media was replaced 4 hours after plating with William’s E media supplemented with serum-free Hepatocyte Maintenance Supplements (Life Technologies, CM4000) or William’s E Maintenance Media supplemented with Hepextend™ Supplement. An overlay of GelRed® Basement Membrane Matrix (Life Technologies, A14132-01) diluted in William’s E Maintenance media was applied 24 hours after plating. Media was changed daily for the duration of cultures. 

P450 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-genacacetin 1; CYP2B6-bupropion; CYP2D6-dextromethorphan; CYP3A4-pentobarbital. After incubation, supernatants were collected and frozen until analysis. Metabolites were detected using LC/MS/MS.

Gene Expression Analysis

Hepatocytes were cultured for 5 days in indicated media. For induction studies, prototypic inducers were added on day 3 of the culture for a total exposure time of 48 hours. RNA was harvested using the PerkinElmer RNA Mini kit (Life Technologies, 12180819A). Five hundred nanograms of RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription kit (Life Technologies, 4368813). Gene expression was assessed using a custom designed TaqMan® Array card containing 48 hepatocyte-specific genes of interest to ADME/Tox studies.

RESULTS

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

Figure 2. Hepextend™ supplementation increases cytochrome P450 activity in plated cryopreserved human hepatocytes

Figure 3. Basal gene expression of Phase I and II drug metabolism enzymes and transporters

Figure 4. Hepextend™ cultures retain inducibility

CONCLUSIONS

- Our Hepextend™ Supplement (50X) allows for long term (5-6 days) culturing of primary human hepatocytes with maintenance of hepatocyte polarity and basal P450 enzymatic activities.
- Future studies will examine the impact of Hepextend™ on analysis of low turnover compounds as well as low dose long term toxicity studies.

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