Human Hepatocyte/Kupffer cell 3D Spheroid Co-cultures: Characterization and Application for DILI Studies

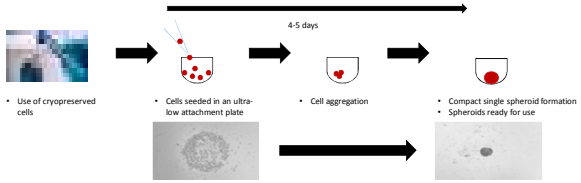
Patrina Gunness,[1] Albert P. Li[2*]
[1]In Vitro ADMET Laboratories, Malden, MA; [2]In Vitro ADMET Laboratories, Columbia, MD

Introduction

- Drug-induced liver injury (DILI), is an unpredicted clinical event that can lead to acute liver failure, and results in the termination of drug development program and the application of regulatory restrictions, including boxed warnings.
- The well documented limitations of current 2D *in vitro* hepatocyte cell culture and *in vivo* animal models does not allow for the accurate prediction of DILI in human. There is an urgent need for improved *in vitro* human hepatocyte models to address these limitations and allow for more accurate prediction of DILI in humans.
- The 3D cell culture of hepatocytes is a rapidly expanding field in an attempt to recreate, in a controlled, artificial environment; the complex 3D microenvironment of the human liver, which is essential for hepatocyte longevity and function. A hepatocyte spheroid culture is a 3D cell culture of organized, aggregates of hepatocytes.
- The goal of the study was to develop a procedure to reproducibly culture human hepatocytes and Kupffer cells as spheroids and to characterize these long-term cultures for their suitability for DILI studies.
- Results show that these long-term spheroid cultures (1) have liver-like morphology and function, (2) are sensitive to prototypical hepatotoxicants, (3) respond to an inflammatory stimulus and (4) can distinguish between hepatotoxic and non-hepatotoxic compounds.
- Taken all together, the data suggest that these long-term spheroid cultures may be a useful tool to study DILI.

Materials & Methods

Culture of human hepatocyte spheroids:

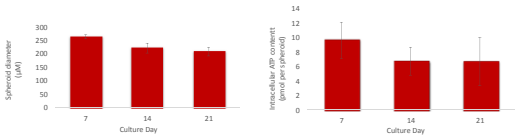


Size assessment: The size of the spheroids was assessed by measuring their diameter, using a stage micrometer. The assessment was performed in replicates of 3-4. The data is presented as the mean ± SD.
Cell viability: Cell viability was assessed by measuring the intracellular ATP content (CellTiter® Glo 2.0 assay, Promega) in spheroids. The luminescence was measured using a plate reader (1420 Multilabel Counter VICTOR^3–V, Perkin Elmer). The assay was performed in replicates of 3-4. the data is presented as the mean ± SD.
Histology: Pooled spheroids were fixed, embedded in paraffin and subsequently subjected to immunohistochemistry (IHC) procedures for H&E, CD68 and Ki67 staining.
Albumin production: Albumin production was assessed in medium supernatant (Human Albumin ELISA, Bethyl Laboratories, Inc.). The assay was performed in replicates of 3. The data is presented as the mean ± SD.
IL-6 production: Spheroids were exposed to LPS (10 µg/mL) for 72 hours. Following the exposure, IL-6 production was assessed in medium supernatant (Human IL-6 ELISA, Boster Biological Technology Co., Ltd.). The assay was performed by pooling medium supernatant from 4 spheroids.
Phase I P450 & phase II enzyme activity: Spheroids were incubated with respective enzyme substrates for 6 hours. Following the incubation period, the medium supernatant was collected and subjected to LC/MS analyses (API 5000 mass spectrometer with an electrospray ionization source (AB SCIEX) connected to Acquity UPLC). The assay was performed in replicates of 3. The data is presented as the mean ± SD.
Short-term cytotoxicity studies: Spheroids were exposed to respective hepatotoxicants for 5 days. Cytotoxicity was assessed by measuring cell viability (as previously described). The assay was performed in replicates of 3. The data is presented as % control. IC_{50} curves were generated using GraphPad Prism® 6. The data is presented as the mean ± SD.

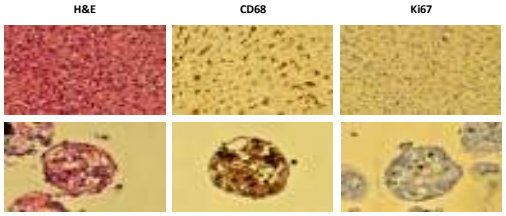
- **trovafloxacin/levofloxacin ± LPS:** Spheroids were pre-treated with LPS (0, 10 µg/mL) for 72 hours, followed by co-treatment with trovafloxacin or levofloxacin for 5 days. Following the exposure period, cytotoxicity was assessed by measuring cell viability (as previously described). The assay was performed in replicates of 3. The data is presented as % control. IC_{50} curves were generated using GraphPad Prism® 6 measured. The data is presented as the mean ± SD.

Results

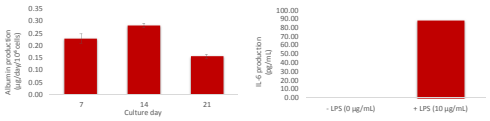
Size and viability of human hepatocyte spheroid cultures



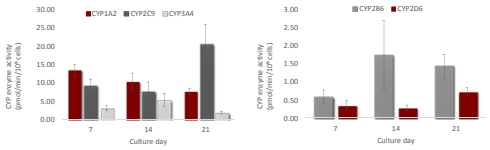
Human hepatocyte spheroid cultures have liver-like morphology



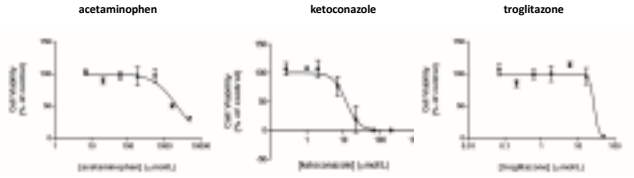
Albumin production **IL-6 production**



Long-term phase I P450 enzyme activity in human hepatocyte spheroids

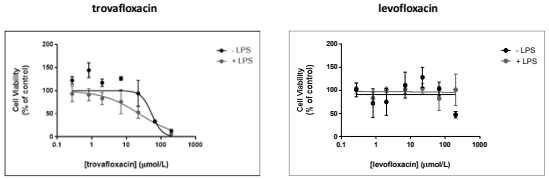


Prototypical hepatotoxicants induce cytotoxicity in human hepatocyte spheroids



IC_{50}: 2219 (1347-3656) µmol/L IC_{50}: 11.85 (8.90-15.79) µmol/L IC_{50}: 27.60 (9.90-76.92) µmol/L

Human hepatocyte spheroids can distinguish between a hepatotoxic and non-hepatotoxic compound



Compound	IC_{50}	
	-LPS	+LPS
trovafloxacin	53.77 (17.96-161.0)	23.43 (15.23-36.06)
levofloxacin	N/A	N/A

Conclusions

- Human hepatocyte spheroid cultures have long-term liver-like morphology & functionality.
- Human hepatocyte/Kupffer cell spheroid co-cultures respond to an inflammatory stimulus; suggesting that it may be useful tool for inflammation-mediated toxicity.
- Prototypical hepatotoxicants induce cytotoxicity in human hepatocyte spheroid cultures.
- Exposure to an inflammatory stimulus increases the sensitivity of hepatocyte/Kupffer cell spheroid co-cultures to trovafloxacin exposure.
- Human hepatocyte spheroid cultures can distinguish between a hepatotoxic and a non-hepatotoxic compound.
- Taken all together, the data suggest that these long-term 3D hepatocyte/Kupffer cell spheroid cultures may be a useful tool for preclinical drug metabolism and toxicity studies, including inflammation-mediated hepatotoxicity.

*Corresponding author: Albert Li (lialbert1@invitroadmet.com))