Drug-induced liver injury (DILI) remains the main cause of acute liver failure and post-marketing drug withdrawals. The well documented limitations of preclinical in vivo animal studies and in vitro two-dimensional (2D) human hepatoctye models hinder the accurate prediction of DILI in humans. The objective of this study was to characterize long-term three-dimensional (3D) co-cultured human liver microtissues (MTs) to assess its suitability for use in drug-induced hepatotoxicity studies. 2D monolayer and 2D co-cultures of cryopreserved primary human hepatocytes (PHHs) and Kupfer cell line were maintained for 2 (2D), 4 (4D), or 4 (4D) weeks and assessed for viability, liver-specific protein, function and mRNA-expression. Results illustrated that the MTs had a longer life span than the conventional 2D cultures and maintained a consistent diameter (250 – 350 μm) in the absence of a necrosis core over the culture period. Albumin secretion by the MTs was initially higher (2.00 ± 0.10 fmol/cell) in the MTs versus the 2D cultures on culture day 7 and was maintained in the MTs over the culture period. Taken together, the results from the study illustrate that the long-term 3D human liver MT cultures have liver-specific phenotype and functionality and may be an accurate in vitro model to study DILI.

Introduction

DILI is a serious health concern and accounts for approximately 50% of acute liver failure cases [Shaw et al. 2010]. DILI is difficult to predict and often occurs during the clinical phases of drug development or post marketing [Shaw et al. 2010; Tuschi et al. 2008]. The well documented limitations of in vitro 3D hepatic models and in vivo animal models hinder the accurate prediction of DILI in humans. The maintenance of the in vivo physiological environment is critical for accurate toxicological responses in vitro [Meng 2010]. In vitro 3D cell cultures may possess in vivo – like cell-cell and cell-extracellular matrix interactions required for normal cell physiology and function [Lin and Chang 2008; Meng 2010]. Currently, there are several 3D in vitro human hepatic models (i.e. isolated whole and perfused livers, perfusion bioassays), however, these systems can be technically challenging, labour intensive and are unsuitable for high throughput applications. Moreover, perfused whole human livers are viable for only 2-3 hours, while human liver slices are viable for up to 3-5 days and therefore, cannot be used for relevant chronic toxicity studies [Van de Beek enkamp et al. 2007]. Therefore, there is a need for high-throughput, long-term in vitro 3D human hepatic models that can accurately predict DILI in humans.

Study objective

The main objective of this study was to characterize long-term in vitro 3D human liver microtissue cultures.

Methods

In vitro 3D human liver microtissues were obtained using InSphero’s hanging drop technology. The human liver microtissues were co-cultures of cryopreserved primary human hepatocytes (n=1) and Kupfer cells (n=1). Cultures were maintained in a sterile environment at 37°C, 5% CO2.

Results

H&E staining, CYP3A4, CD68 and BSEP protein expression in human liver microtissues

Culture day 7 Culture day 14 Culture day 21 Culture day 28

Figure 3. H&E staining, CYP3A4, CD68 and BSEP protein expression in 3D human liver microtissues. Pooled 3D human liver microtissues were collected on culture days 7, 14, 21 and 28 and subsequently subjected to immunohistochemical staining for the respective proteins.

mRNA expression of transporters, phases I and II ADME genes in 3D human liver microtissues

Phase I ADME genes Phase II ADME genes

Figure 4. The mRNA expression of transporters, phases I and II ADME genes in 3D human liver microtissues. RNA from 2D monolayers (culture day 7) and 3D cultures (culture days 7, 14, 21 and 28) was subjected to transcriptomic analysis (Affymetrix GeneChip® Human Genome 2.0 ST arrays). Gene lists were subsequently generated using the Ingenuity Pathway Analysis software. The data is presented as the fold change in mRNA expression of the respective genes in 3D human liver microtissue cultures (culture days 7, 14, 21 and 28) compared to 2D monolayer cultures (culture day 7).

ATP content in 3D human liver microtissues

Figure 1. Intracellular ATP content in 3D human liver microtissues. Intracellular ATP content was assessed using Ultrasensitive AlamarBlue® assay, Promega, USA) in 3D human liver microtissues over the culture days 7, 14, 21 and 28. The assay was performed in triplicates. The results are presented as the mean ± SD.

Albumin production in 2D monolayers and 3D human liver microtissues

Figure 2. Albumin production in 2D monolayers and 3D human liver microtissues. Albumin production was assessed (Human Albumin ELISA assay, Beyh etel laboratories inc., USA) in 2D monolayers and 3D human liver microtissues over the respective culture period. The assay was performed in triplicates. The results are presented as the mean ± SD.

CYP enzyme activity in 3D human liver microtissues

Figure 5. The enzyme activities of CYPs 1A2, 3A4 and 2D6 in 3D monolayers and 3D human liver microtissues. The enzyme activities of CYPs 1A2, 3A4 and 2D6 were assessed in 2D cultures (culture day 2) and 3D cultures (culture days 7, 14, 21, 28) following incubation with Phenacem (CYP1A2), Midazolam (CYP3A4) or Bufalin (CYP2D6) for 24 hours. Following the respective incubation period, the media from 2D monolayer cultures or 3D human liver microtissue cultures were collected and subsequently subjected to LC/MS analyses (Pharmaceuticals GmbH, Germany). The assay was performed in replicates of 3 (2D cultures) or 6 (3D cultures). The results are presented as the mean ± SD.

References