Human liver microphysiological system for studying acute and chronic drug-induced liver toxicity *in vitro*

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INTRODUCTION

The liver is one of the organs most susceptible to drug toxicity and drug-induced liver injury (DILI). DILI is a major cause of drug attrition, with more than 750 FDA approved drugs known to have a degree of DILI risk [1]. There are a number of strategies to de-risk DILI in drug discovery and, in recent years, focus has turned to human *in vitro* 3D liver models to better predict DILI in early and pre-clinical development [2]. These models culture primary human liver cells, often in co-cultures, in a physiologically relevant environment allowing them to stay functional for extended periods of time.

Here, we assessed whether a microphysiological system (MPS), also known as Organ-on-a-Chip (OOC), *in vitro* liver model could be used to understand the detailed mechanistic aspects of liver toxicity. The MPS has previous been shown to maintain highly functional 3D liver microtissues, under flow, for up to 4 weeks [3], potentially making it highly suited to assessing DILI. We used two antidiabetic thiazolidinediones, troglitazone (was market approved and later removed due to DILI) and pioglitazone (approved medicine but with known DILI risk) to assess whether the MPS can detect acute and chronic toxicity. DILI by both of these compounds is typically difficult to detect using standard *in vitro* liver assays and *in vivo* pre-clinical models [4]. For each compound, a range of functional liver-specific endpoints were analysed (inc. clinical biomarkers) across a concentration response to generate EC50 curves. Functional liver-specific endpoints were analysed to create a distinct mechanistic "signature of hepatotoxicity" from the MPS to demonstrate its capacity to assess human DILI risk of novel agents.

AIMS

1. Demonstrate the MPS platform capacity to generate EC_{50} curves for six toxicological endpoints for a known toxicant, troglitazone.
2. Demonstrate that the MPS platform can detect DILI responses from a mild toxicant – pioglitazone.
3. Demonstrate how using multiple endpoint analyses enables the generation of a hepatotoxicity signature.

MATERIALS AND METHODS

Cryopreserved primary human hepatocytes (PHH) were obtained from BioIVT (Europe). 0.6 x 10^6 PHH were seeded into each well on a PhysioMimix™ OOC LC-12 plate (CN Bio Innovations) in seeding medium (William's E Medium, 3.6% Cocktail A, 5% FBS, 1 µM Dexamethasone). Media was changed at Day 1 with maintenance medium (William's E Medium, 4% Cocktail B and 1 µM Dexamethasone). The cells were cultured in the PhysioMimix™ platform for up to 2 weeks.

At Day 4, each compound was dosed at 7 concentrations in WEM maintenance medium containing 0.1% DMSO, and then every 48 hours for 8 days. Vehicle control consisted of WEM maintenance medium containing 0.1% DMSO. Treated and untreated wells were randomized throughout the plates, and each condition was tested in triplicate.

Production of albumin was measured by ELISA (R&D systems), LDH release was quantified using the Cyto-tox96 assay (Promega), urea synthesis was quantified using QuantiChrom™ kit, CYP3A4 activity was measured using P450-Glo™ CYP3A4 Assay (Promega), ALT activity was measured with Alanine Transaminase Activity Assay kit (Abcam), and cell viability was assessed using the CellTiter-Glo® 3D Cell Viability assay (Promega). Compounds were procured from Tocris Bioscience.

RESULTS

Figure 1 – Liver MPS model maintains 3D human liver microtissues
A) The *in vitro* model utilises the PhysioMimix™ OOC Microphysiological System, which uses open well plates designed for the culture of primary liver cells in 3D in an engineered scaffold. B) Schematic representation of an LC12-MPS liver-on-chip plate, scaffold and liver 3D microtissue formed within its microchannels. C) Evidence of formation of 3D polarized liver microtissues recreating the liver microarchitecture.

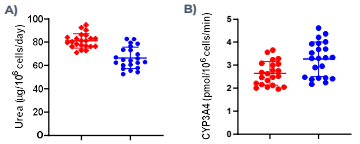

Figure 2 – Liver MPS produces highly consistent and highly function liver microtissues
Before dosing with compound, liver microtissues were assessed for A) Urea synthesis and B) CYP3A4 activity. Red dots - cultures prior to treatment with troglitazone, and blue dots - cultures prior to treatment with pioglitazone. Data shown also includes mean values ± SD (N=24). Urea QC pass range = 40-100 ug/10^6 cells/day. CYP3A QC pass range = 1-5 pmol/10^6 cells/day.

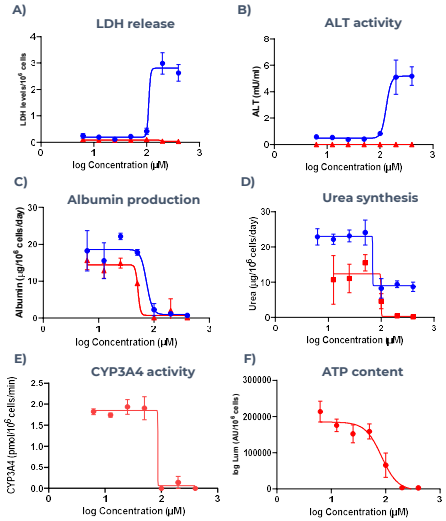

Figure 3 – Determining EC_{50} concentrations for multiple hepatotoxic endpoints following acute and chronic exposure to troglitazone.
Liver microtissues exposed to compared and analysed for A) LDH release, B) ALT release, C) Albumin production, D) Urea synthesis, E) CYP3A4 activity, F) ATP content. Blue lines – acute exposure (48 hrs), red lines – chronic exposure (192 hrs). All endpoints are measured from the same liver MPS cultures. Data shown are mean ± SD, N=3.

Assay	Troglitazone				Pioglitazone			
	48 hrs		192 hrs		48 hrs		192 hrs	
	EC_{50}	R^2	EC_{50}	R^2	EC_{50}	R^2	EC_{50}	R^2
LDH	110	0.98	ND		ND		ND	
ALT	131	0.95	ND		ND		ND	
Albumin	73	0.93	51	0.93	69	0.80	87	0.72
Urea	70	0.93	100	0.74	86	0.73	ND	
CYP3A4	-	-	87	0.98	-	-	265	0.61
ATP	-	-	84	0.92	-	-	348	0.69

Table 1 – Liver microtissues detect toxicity of troglitazone and pioglitazone.
Liver microtissues exposed to both compounds for 48 or 192 hours. ND = data not plottable as EC_{50} curve. Line = not assayed. Data shown are generated from seven-point dose responses, with N=3 per concentration.

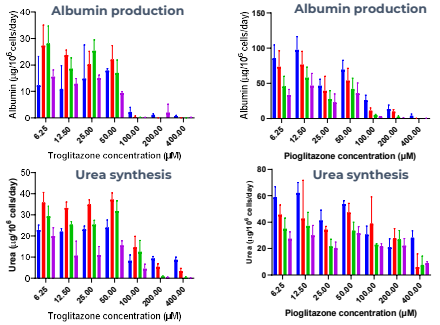

Figure 4 – Measuring hepatic biomarkers over time following exposure to troglitazone and pioglitazone demonstrates acute and chronic toxicity effects.
Liver microtissues were exposed to troglitazone and pioglitazone for 48 hrs (blue), 96 hrs (red), 144 hrs (green) and 192 hrs (purple). Cell culture medium was refreshed every 48 hours and analysed for albumin and urea synthesis. Data shown are mean ± SD, N=3.

CONCLUSIONS

The acute and chronic exposure of troglitazone and pioglitazone were investigated using a Liver MPS model. Functional and cell health endpoints were used create a distinct mechanistic "signature of hepatotoxicity" and demonstrate the ability of the MPS to predict DILI risk. The inclusion of ALT, a routinely measured clinical marker, within the panel of endpoints enables a direct comparison to be made between in vitro data and in vivo outcomes. Troglitazone caused a clear acute toxic response, Cmax driven, that was detected by ALT and LDH release and a rapid reduction in albumin and urea production at circa 15x Cmax. Cellular endpoints (CYP activity and ATP content) further confirmed troglitazone toxicity, generating highly comparable EC50 values from all assays. This data mirrors that of alternative advanced 3D in vitro models which report similar EC50 values for troglitazone, and out-performs standard in vitro PHH culture where this DILI-inducer remains undetected [4].

Both acute and chronic Pioglitazone toxicity effects were detected. No LDH or ALT release was detected, however, after 48 hrs a mild reduction in albumin and urea was reported at circa 25 x Cmax. The effects on albumin persisted for 192 hrs, whilst a reduction in CYP activity and ATP content was observed at high Pioglitazone concentrations, circa 100 x Cmax. Together, these results demonstrate the ability of the liver MPS to detect the toxicity of compounds with mild DILI concern. This is superior to other advanced in vitro liver models where the toxicity of pioglitazone is not observed [4]. The data demonstrates the value of measuring a range of endpoints to produce a "signature of hepatotoxicity", as by doing so, drugs with differing level of DILI concern can be identified (including compounds not detectable by other in vitro methods) and the mechanisms of that toxicity revealed. By incorporating this liver MPS model into drug development work flows it will enable mechanisms of DILI to be better understood and help to reduce drug attrition by identifying earlier in the development process (before clinical trial initiation) compounds with human-specific DILI concern.

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