Assessing drug-induced liver injury using a sensitive and selective human liver microphysiological system and clinical biomarkers

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Introduction

Drug-induced liver injury (DILI) is the most common cause for acute liver failure in the USA and Europe. It is also a determining factor for drug attrition during development process, with over 750 FDA-approved drugs currently known to potentially cause different levels of DILI severity in humans [1].

Over the recent years, focus has turned to human in vitro 3D liver models to provide solutions to the limitations of standard in vitro cell culture models in detecting chronic drug exposure [2].

However, all these approaches still have drawbacks, and culturing Primary Human Hepatocytes (PHHs) in a more physiologically relevant microenvironment could provide higher levels of functionality for extended periods of time; thus enabling the investigation of prolonged exposure to potential hepatotoxicants. Additionally, to improve the translational relevance of any advanced in vitro liver culture, clinically-relevant functional endpoints or toxicity output biomarkers must be utilized to allow data to be compared in vivo or clinical scenarios [3].

Here, we describe a human liver microphysiological system (MPS), comprised of human primary liver parenchymal and non-parenchymal cells cultured in 3D microtissues on an engineered scaffold under perfusion. The methodology was validated with a set of 15 compounds of which 13 severely and mildly hepatotoxic small molecules and two antisense oligonucleotides (ASOs).

Aim

Demonstrate the capability of a human liver MPS to generate clinically relevant, high-content data (six endpoint EC_{50} curves) from a broad set of 15 compounds of which 13 are known severely and mildly hepatotoxic pharmaceutical drugs, and two ASOs.

Material & Methods

Cryopreserved PHHs were obtained from BioIVT and Human Kupffer Cells (HKCs) were purchased from Lonza. Co-cultures of PHHs (0.4 x 10^6) and HKCs (0.04 x 10^6) were seeded into PhysioMimix™ Liver Plates. Cells were cultured in the PhysioMimix Organ-on-a-chip (OOC) System for eight days in Advanced DMEM Medium, 4% Cocktail B and 500 nM hydrocortisone.

At day four, liver microtissues were dosed with seven increasing concentrations of each test compound. Microtissue dosing was repeated every 48 hours for four days. Vehicle control consisted of Advanced DMEM maintenance medium containing 0.1% DMSO, while the positive control consisted of 100 µM chlorpromazine. Treated and untreated wells were randomized throughout the plates, and each condition was tested in triplicate.

Albumin production was measured by ELISA (R&D systems), LDH release using the Cyto-tox96 assay (Promega), urea synthesis using QuantiChrom™ kit, CYP3A4 activity 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).

Results

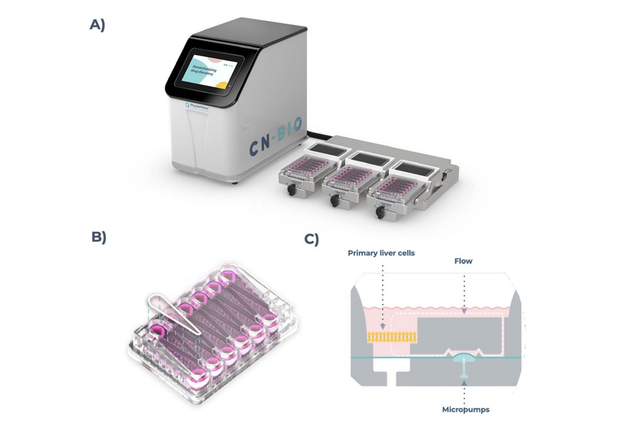

Figure 1 – The PhysioMimix™ OOC enables the generation of 3D human liver microtissues, functional for up to four weeks. A) The PhysioMimix OOC is comprised of a controller, docking station and MPS drivers into which MPS plates are inserted. B) Schematic representation of a PhysioMimix Multi-chip Liver plate, which features an open well design for the co-culture of PHHs and HKCs in 3D on an engineered scaffold. C) Cross-section of an in individual liver MPS well.

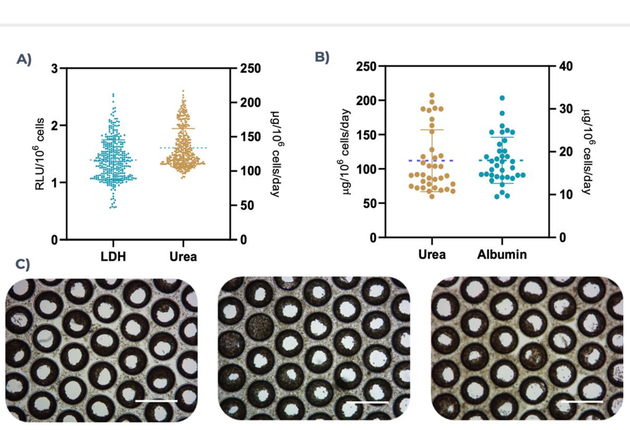

Figure 2 – The Liver MPS produces highly reproducible data and consistent microtissues. A) 3D Liver microtissue quality control (QC) metrics at day four (Mean ± SD, N = 360), B) Functionality assessment at the end of the drug dosing (96 hrs). Data was collected from 12 individual experiments; for each experiment 3 vehicle control replicates were used (data shown Mean ± SD, N = 36). C) Phase contrast microscopy of healthy control liver microtissues after a eight-day culture, from three independent experiments (magnification 10x). Scale bar = 350 µm.

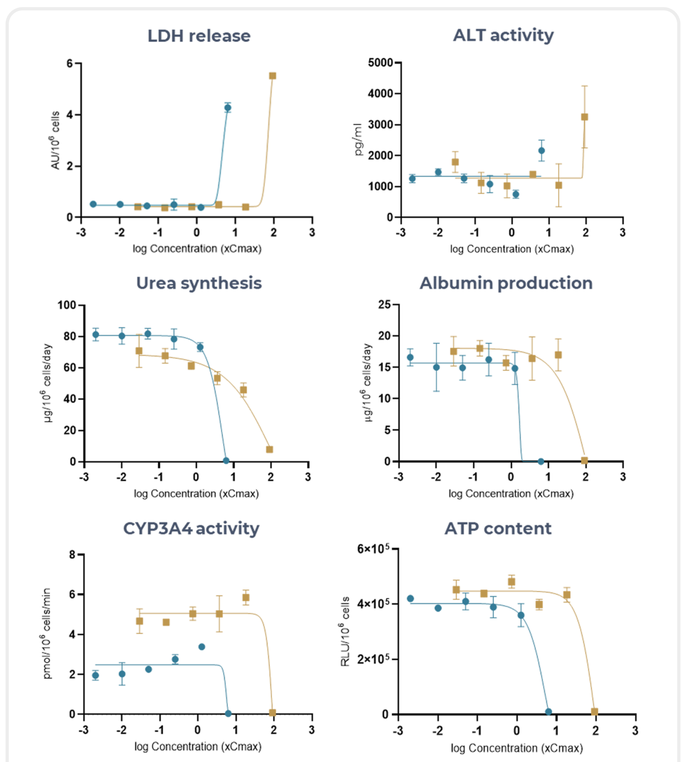

Figure 3 – Liver MPS accurately generates DILI profiles of tolcapone (high-DILI-concern) and entacapone (low-DILI-concern), using multiple hepatotoxic endpoints. Liver microtissues were exposed to tolcapone (blue) and entacapone (brown) for 96 hrs. Endpoint measurements were all derived from the same liver MPS culture. Data shown are mean ± SD, N = 3 and all from 96hrs samples apart from LDH release which was measured at 48 hrs.

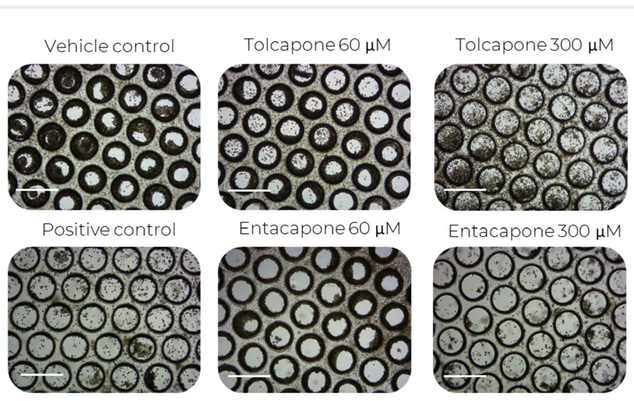

Figure 4 – Toxic effect of tolcapone (high-DILI-concern) and entacapone (its less hepatotoxic structural analogue) on liver microtissues. Representative phase contrast microscopy of untreated and treated liver microtissues after an eight-day culture (magnification 10x). Scale bar = 350 µm.

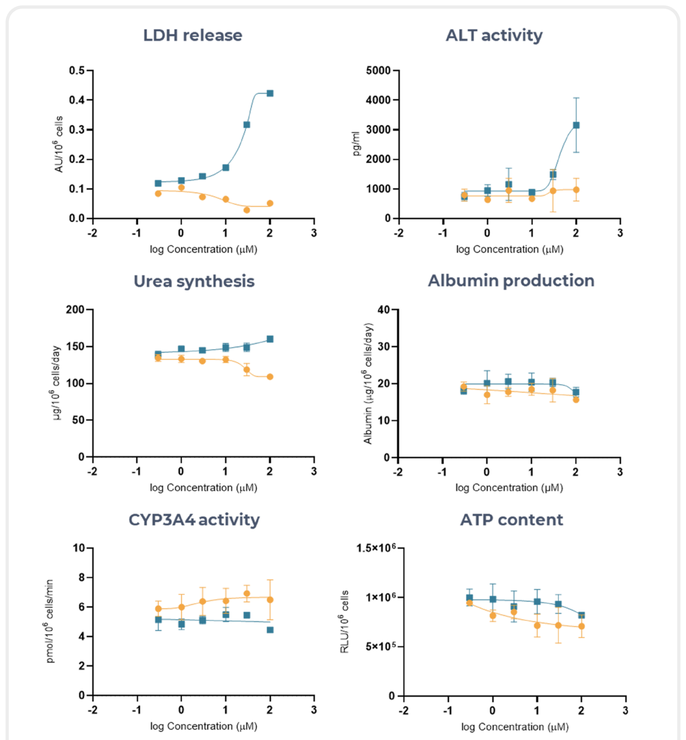

Figure 5 – Liver MPS accurately determines the DILI risk of two ASOs (LNA43, LNA32), using multiple hepatotoxic endpoints. Liver microtissues were exposed to LNA43 (blue) and LNA32 (brown) for 96 h. Endpoint measurements were all derived from the same liver MPS culture. Data shown are mean ± SD, N = 3 and all from 96h samples.

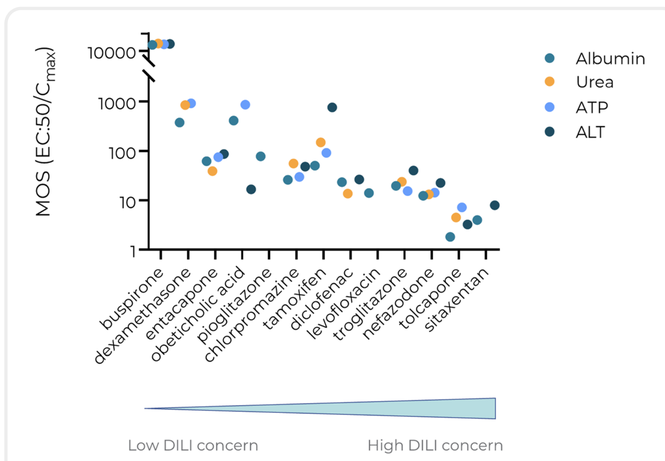

Figure 6 – MPS DILI assay accurately determines the liability of a broad set of known severely and mildly hepatotoxic compounds. Exposure-corrected cytotoxicity or margin of safety (MOS = EC:50/Cmax) were determined for 4 key biomarkers albumin, urea, ATP, and clinical biomarker ALT, following 96 hrs of exposure. Endpoint measurements were all derived from the same liver MPS culture. Data shown are mean ± SD, N = 3. Tested compounds are arranged based on DILI-rank, from low DILI concern (left) to high DILI concern (right).

Conclusions

The liver MPS produces highly consistent and functioning liver microtissues. The data presented in **Figure 2A** are generated from a large number of experiments and shows good levels of reproducibility with low intra- and inter-study variability. After an eight-day culture, multiple health and hepatic metrics (albumin, urea, CYP3A4, ATP) were assessed in healthy control microtissues and demonstrated high levels of hepatic functionality and reproducibility of formed microtissues in the scaffolds (**Figure 2B, C**).

Tolcapone (high-DILI-concern) caused a clear acute toxic response, C_{max} driven, that was detected by clinical biomarker ALT and LDH release (4.7 x C_{max}) and a rapid reduction in albumin and urea production at approx. 5 x C_{max} (**Figure 3**). Chronic exposure was captured by all tested endpoints, particularly in albumin at just 0.3 x C_{max}. Cellular endpoints (CYP activity and ATP content) further confirmed tolcapone toxicity, with highly comparable EC_{50} values from both assays. In contrast, the model did not detect acute and chronic toxicity effects following treatment with tolcapone's non-toxic structural analogue, entacapone (**Figure 3, 4**). Moreover, the liver MPS model distinguished between safe and toxic ASOs (LNA32 – safe in vivo, LNA43 – severely hepatotoxic in vivo), with acute toxic response that was detected only by clinical biomarker ALT and LDH release at 96 h of exposure (**Figure 5**). These findings recapitulate the published data from in vivo trials conducted on mouse and rat models [4, 5].

The liver MPS accurately predicted human DILI for all 15 tested compounds (**Figure 6**). Due to its enhanced sensitivity, the model was able to identify hepatotoxicants (pioglitazone, dexamethasone, levofloxacin) that were not detected using 2D and other 3D in vitro models. The data and experimental approaches demonstrated how the liver MPS model can be used reliably and consistently, producing highly functional and metabolically active liver microtissues. These liver microtissues can be used routinely in DILI screens to accurately predict the liability of novel compounds. Incorporation of these methodologies into pharmaceutical workflows will enable for rapid identification of compounds with human DILI liabilities leading to significant economic savings and early de-risking of potentially unsafe compounds.

References

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