A human liver microphysiological system for assessing mechanisms of toxicity during drug development

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Introduction

Drug-induced liver injury (DILI) remains the most common cause for acute liver failure in the USA and Europe and is a leading cause of attrition of compounds in drug development. The current models used in drug development and preclinical safety testing, although sufficient at capturing most intrinsic toxic events, have significant limitations and are not effective at predicting or understanding more complex DILI mechanisms in humans. Microphysiological systems (MPS), otherwise known as organ-on-a-chip (OOC), provide a solution to this challenge by enabling the long-term culture and in-depth examination of highly human relevant liver microtissues. Moreover, these models provide translational data generated from key clinical biomarkers, enabling a route to more effective and predictive drug development. In this study, we utilized the high content data output capability of the PhysioMimix® Human DILI assay and kit to understand the mechanism of toxicity of known tool compounds. We highlight the advantage of using the approach to derive human translatable mechanistic insights ahead of more costly drug development phases.

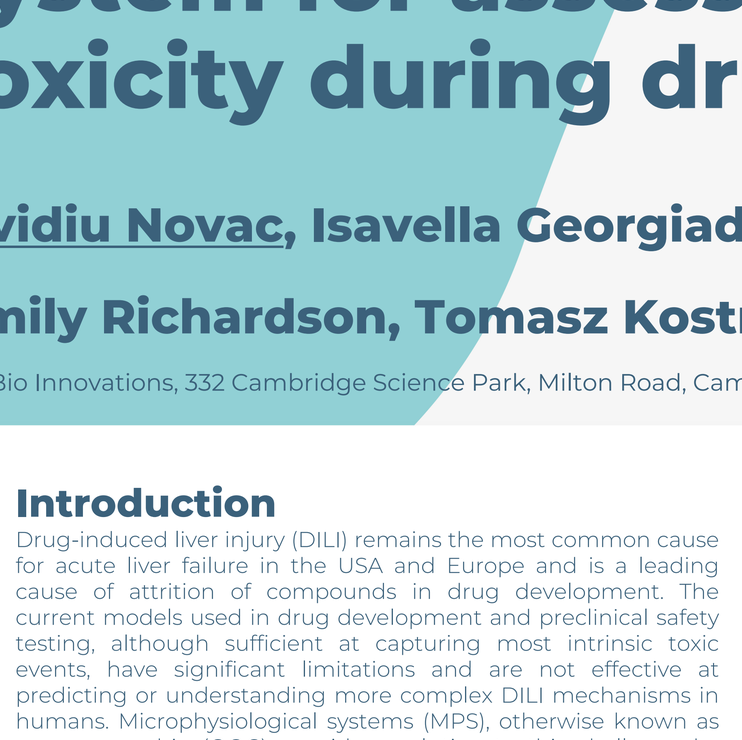

Figure 1. The PhysioMimix® DILI assay accurately determines the risks of a broad set of known severely and mildly hepatotoxic compounds. Exposure-corrected cytotoxicity or margin of safety (MOS = EC:50/C_{max}) were determined for four key biomarkers albumin, urea, ATP, and clinical biomarker ALT, following 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).

Materials and Methods

Using the PhysioMimix DILI Assay Kit: Human 24, Primary Human Hepatocytes (PHH) and Human Kupffer Cells (HKCs) were seeded at a 10:1 ratio in PhysioMimix Multi-chip Liver-12+ plates and cultured under perfusion using the PhysioMimix Organ-on-a-Chip (OOC) System for 14 days (Figure 2).

At day four, The PhysioMimix Liver MPS was subjected to 10-day exposure with a range of tool compounds recommended by the IQ MPS Consortium for DILI validation. An equivalent dose to clinical concentration and 1.5x the Minimal Important Difference (MID) clinical concentration doses were tested, and vehicle control was 0.1% DMSO. Treatments were randomised across the plates and each condition tested in triplicate. Liver function was assessed for a broad spectrum of liver-specific endpoints on the cellular structures and culture medium. To perform a comprehensive mechanistic evaluation of DILI events, oxidative stress, mitochondrial dysfunction, steatosis, dysregulation of bile acid synthesis or transport, inflammatory response were investigated using a wide range of clinically relevant biomarkers.

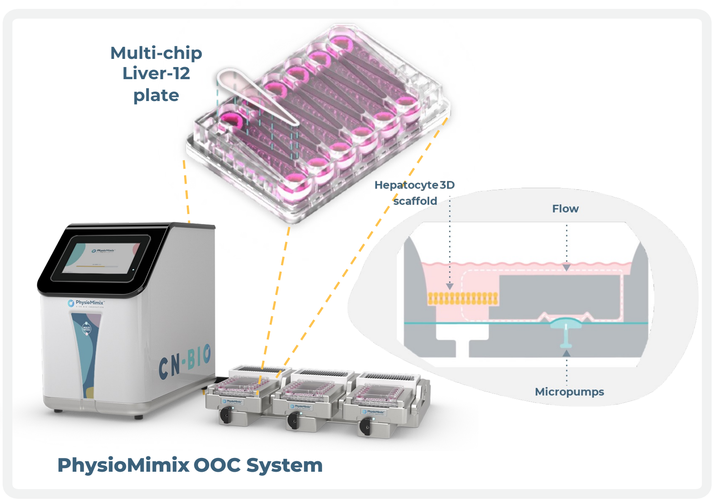

Figure 2. The MPS model forms functional 3D human liver microtissues. (A) Schematic representation of PhysioMimix Liver-12 plate, including cross-section of one open cell culture well indicating the 3D scaffold and fluidic flow.

Results

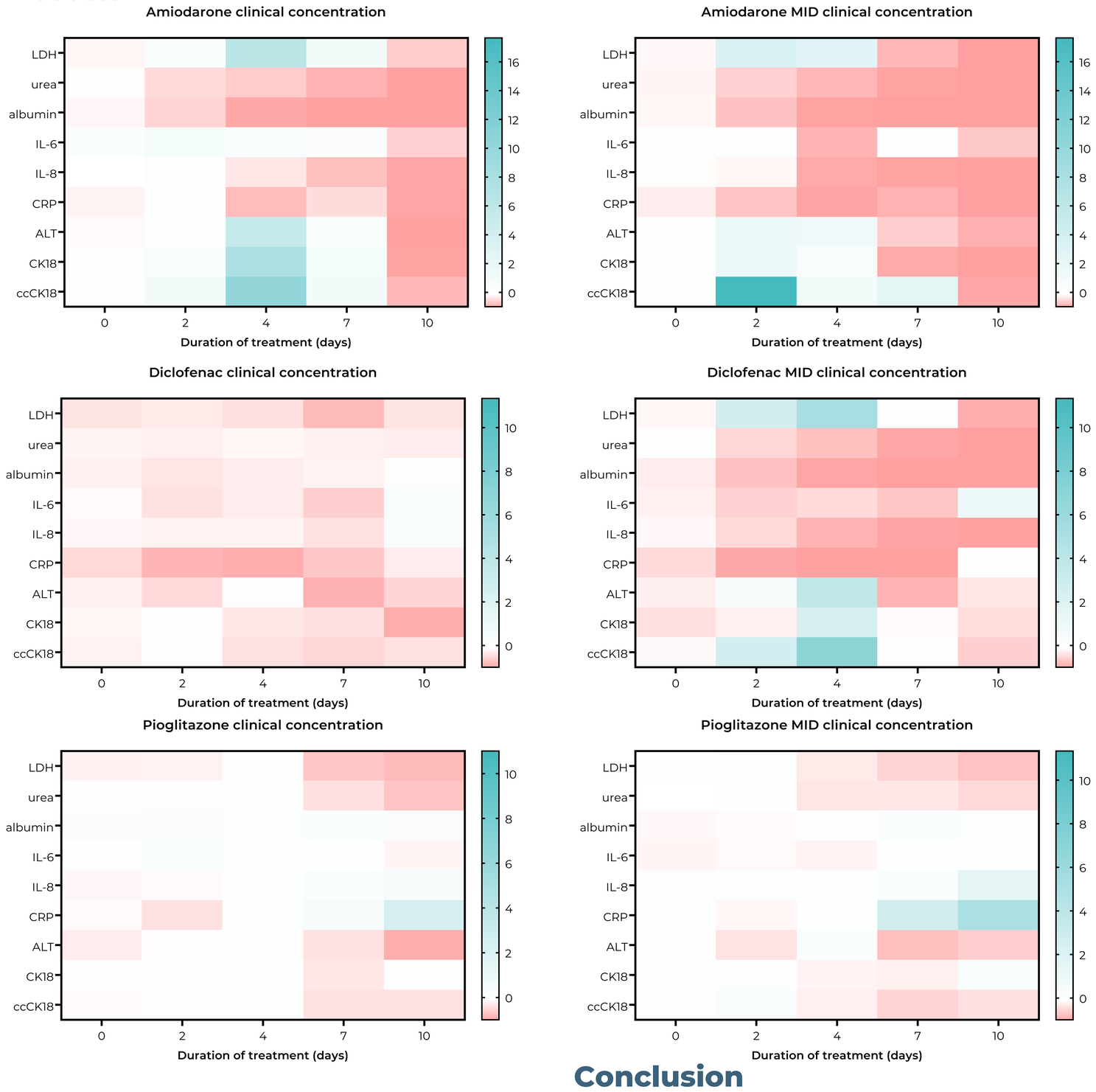

Figure 3. The PhysioMimix DILI assay captures "signature of toxicity" in liver microtissues. Liver microtissues were treated once-daily with amiodarone, diclofenac and pioglitazone for 10 days at two concentrations. Data shown are fold-change to vehicle control, determined for each measured time-point, N=3.

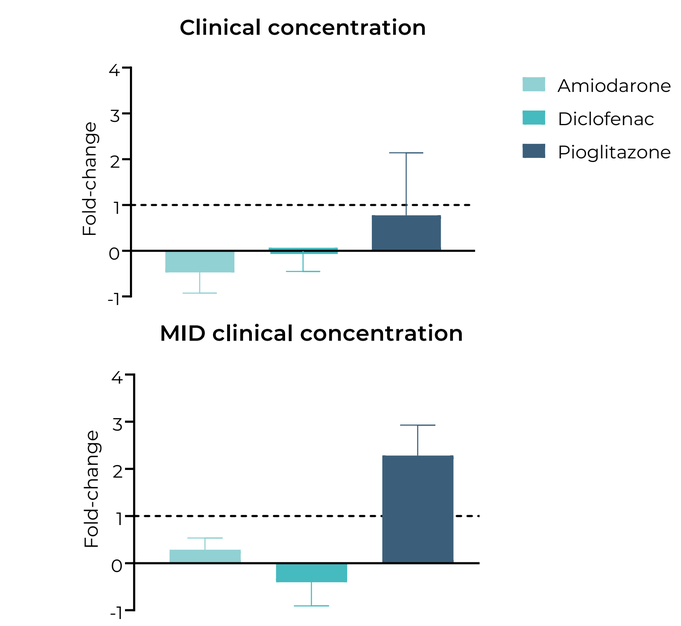

Figure 4. The PhysioMimix DILI assay captures changes in bile acid released. Liver microtissues were treated with amiodarone, diclofenac and pioglitazone at two concentrations for 10 days. Data shown are expressed as fold-change relative to vehicle control, mean ± SD, N = 3, and all measured after 10 days of treatment.

Conclusion

The results of this study demonstrate the ability of the PhysioMimix DILI assay to profile mechanisms of toxicity that cannot be typically observed using standard *in vitro* models. Amiodarone is known clinically to induce increased ALT in patients, without inflammation, and typically damages lysosomes which triggers apoptosis. This is clearly captured in the assay, where significant increases in ccCK18 are detected. At clinical concentration, diclofenac is broadly detected as safe and shows anti-inflammatory effects as per its clinical NSAID role. At MID concentration, increases in LDH, ALT and cytokeratins are detected in the DILI signature, again correctly predicting the clinical hepatocellular injury seen in the clinic. Pioglitazone was demonstrated to show a safe signature, with only mild decreases in urea compared to the control. Collectively, this demonstrates the ability of the assay to better inform decisions to modify drug design, aid clinical translatability and de-risk the clinical progression of drug candidates by providing detailed mechanistic understanding of toxicity. Furthermore, the all-in-one DILI Assay Kit: Human 24 now allows easy access to the PhysioMimix DILI assay through provision of pre-validated cells, Liver-12+ plates, specified and optimized media, plus SOP. Together, this approach enables more efficient and confident drug development and safer medicines for the future.

References

1 – K. Fabre et al., Introduction to a manuscript series on the characterization and use of microphysiological systems (MPS) in pharmaceutical safety and ADME applications, Lab Chip, 2020,20, 1049-1057.
2 - Drug Induced Liver Injury Rank (DILIrank) Dataset, FDA
2 – LiverTox, Clinical and Research Information on Drug-Induced Liver Injury, Bethesda (MD), 2012.
3 -Ewart, L., Apostolou, A., Briggs, S.A. et al. Author Correction: Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. Commun Med 3, 16 (2023)

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