Charaterization and application of 3D Multi-donor Human Liver Microtissues for predictive DILI testing

Karin Roessger, Monika Kijanska and Simon Messner
InSphero AG, Schlieren, Switzerland

Introduction

Utilizing pooled hepatocytes from multiple donors offers the advantage of capturing a more diverse genetic background in a single assay to help mitigate potential bias of single-donor hepatocytes due to individual cytochrome P450 polymorphisms. By pooling hepatocytes from 5 male and 5 female donors, Multidonor Human Liver Microtissues thus more closely approximate the average human response, and extend donor lot availability to ensure a long-term supply of donor material. Available as cocultures with Kupffer cells, or as hepatocyte monocultures, multiple microtissues are ideal for in vitro DILI detection and prediction, compound toxicity screening, mechanistic toxicology studies, and DMPK.

Microtissue Morphology and Functionality

Figure 1: Long-term maintenance of liver-specific morphology via immuno-histochemistry. Multidonor Human Liver Microtissues maintain compact structure (H&E), hepatocyte differentiation by expression of transporters (BSEP), and presence of CDS8 positive Kupffer cells (CD68 for at least 28 days in culture). Tissues were fixed with Carnoy solution and antibodies were detected via IIFDAB.

Figure 2: Long-term maintenance of cell viability, hepatocyte differentiation, and microtissue size. Multidonor Human Liver Microtissues maintain cell viability (ATP: Promega CellTiter-Glo®) and microtissue size for at least 28 days (follow-up).

Compund Screening for Drug-Induced Liver Injury

Figure 3: Long-term basal cytochrome P450 activity in Multidonor Human Liver Microtissues. Multidonor microtissues were able to maintain hepatocytic functionality in order to express activity of cytochrome P450 enzymes for at least 28 days. CVF24 samples were incubated at the indicated culture time for 24 hours on 24 wells. Single microtissue supernatant (n=8) were analyzed for presence of substrate metabolites.

Model of Inflammation-mediated Hepatotoxicity

Figure 4: Cytochrome P450 induction activity in Multidonor Human Liver Microtissues. Multidonor microtissues are able to induce activity of CYP4A11 and CYP2A6 when treated with phenobarbital and CYP2J2 when treated with Ompraz. All three conditions showed increased metabolic production when prefatreated with inducers. Microtissues were incubated with inducers for 22 hours with daily re-doing, before addition of corresponding substrate for 24 hours.

Figure 5: Dose-response curves of Multidonor Human Liver Microtissues following treatment with eight different compounds in increasing dosing concentrations. Human liver microtissues were treated for 14 days with eight different compounds, from mild to severe clinical DILI. Accordingly, the margin of safety (MOS) appears to be lower for Stavastain. Tocapore and Enzalutamide are structurally related compounds, which showed also different risk for DILI in the clinic. This difference in margin of safety was recapitulated in Multidonor Human Liver Microtissues. Troglitazone was also correctly predicted as hepatotoxic, while Rosiglitazone was not correctly predicted as true DILI compound, inside the model. It is currently unknown whether troglitazone is the fact that the primary hepatocytes used to produce the microtissues were collected from several different donors with different genetic backgrounds, leading to a population effects where troglitazone could be masked by the function of other cell donors.

The Multidonor Human Liver Microtissues were found to predict a significant hepatotoxic effect in 5 of 6 of the compounds. Rosiglitazone failed to induce a strong hepatotoxic effect in the microtissues over the course of study.

Figure 6: Top: Toxicity testing of compounds rated according to likelihood of causing impairment of liver function (DILI) in humans. Eight compounds were tested on Multidonor Human Liver Microtissues (Figure 5 data). These compounds have been categorized by potential DILI severity according to clinacallat (T: severe DILI 5, m: mild DILI). Top concentration and CIs values were found in vivo studies. MOS values from data shown in Figure 3 of 8 compounds were successfully predicted to have hepatotoxic effects or not as defined by Margin of Safety (MOS) score.

Figure 7: Idiosyncratic toxicity of Multi-donor Human Liver Microtissues with NPCs from different donors. Human liver microtissues were treated with Trovaflaxacin in increasing concentrations, with or without UPS. Viability was assessed following dosing with ATP (Promega CellTiter-Glo®). All three donors had slightly different metabolic profiles, demonstrating phenotypic differences between them. However, all three showed increased sensitivity to Trovaflaxacin when treated with UPS. This shows increased sensitivity of the human liver microtissues within an individual hepatocyte.

Summary and Conclusion

- Stable morphology with expression of bile canaliculi and dense cellular contacts
- Viability for at least 4 weeks in culture
- Functionality of CYP3A4/5 and CYP2B6, CYP2C9, CYP2C19 and CYP2D6 throughout the culture period
- Inducible CYP3A4 and CYP2B6 activity upon Phenobarbital treatment, CYP1A2 activity upon Onemprazol
- Long-term toxicity testing for 14 days with 88% correct prediction of tested non-DILI and DILI compounds
- Inflammation-mediated toxicity of Trovaflaxacin with different Kupffer cell donors, donospecific cytokine profile observed
- Multidonor Human Liver Microtissues represent an advanced, organotypic liver model suited for predictive DILI testing and other liver-related applications.

InSphero AG ● Wagstrasse 27, CH-8952 Schlieren, Switzerland ● Phone: +41 44 515 04 90 ● info@insphero.com ● www.insphero.com