Liver-Chip Model for Determination of Species Differences and Risk Assessment of Hepatotoxicity in Humans

Contributors: Kyung-Jin Jang*[1], Lorna Ewart*[2], Monicah Otieno*[3], Konstantia Kodella[1], Janey Ronxhi[1], Debora Petropolis[1], Abhishek Srivastava[2], Linda C. Andersson[4], Kim Maratea[5], Dominic Williams[2], Monica Singer[3], Jonathan Rubins[1], Gauri Kulkarni[1], Barry Jones[2], Damir Simic[3], Jose Silva[3], Shannon Dallas[3], Peggy Guzzie-Peck[3], Katia Karalis[1], Donald E. Ingber[6], and Geraldine A. Hamilton[1]

[1]Emulate Inc. Boston, USA, [2]AstraZeneca, IMED Biotech Unit Drug Safety and Metabolism, Cambridge, UK, [3]Janssen Pharmaceuticals, Spring House, USA, [4]AstraZeneca, IMED Biotech Unit Drug Safety and Metabolism, Gothenburg, SE, [5]AstraZeneca, IMED Biotech Unit Drug Safety and Metabolism, Waltham, USA, [6]Wyss Institute, Boston, USA

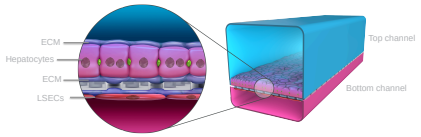
D.E.I. holds equity in Emulate Inc. and chairs its scientific advisory board. A.D.v.d.M serves as a scientific consultant to the company. K.J.J. is an employee of Emulate, Inc., and holds employee stock options

1360/P458

Introduction

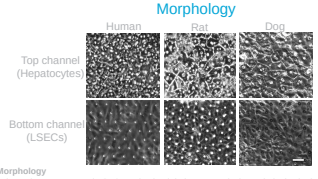
Drug-induced liver injury (DILI) remains a major cause of drug attrition during drug discovery and development because animal models and existing in vitro models often do not predict outcome in humans. There is a significant need for more predictive models for DILI. These models must include the relevant cell types that are representative of in vivo tissue, allow for the expression of hepatic functions that recapitulate in vivo metabolic capabilities, provide the ability to conduct long-term maintenance of cell viability to enable repeated drug exposures, and include the capability to demonstrate the diverse mechanisms of DILI. Advanced engineering fabrication techniques were applied to achieve a high level of control over the liver tissue microenvironment. The Liver-Chip incorporates relevant cell-extra cellular matrix (ECM) interactions, a hepatocyte and liver sinusoidal endothelial cell interface, along with relevant cyto-architecture and physiological flow. In addition to the human Liver-Chip, rat and dog models were developed to enable characterization of species differences with respect to pharmacokinetics, toxicity, and mechanism of action.

Liver-Chip

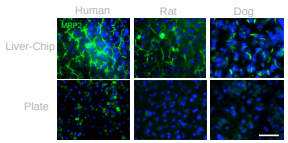


To construct the Liver-Chip, we used the S-1 Chip from Emulate, Inc., which is made of polydimethylsiloxane (PDMS) and contains an upper channel (1 mm high × 1 mm wide) and a lower channel (0.2 mm high × 1 mm wide) that are separated by a porous PDMS membrane that is coated by hepatic extracellular matrix (ECM). In our design, primary hepatocytes were cultured in the upper channel and on the top of the ECM-coated membrane, and primary liver sinusoidal endothelial cells (LSECs) were cultured in the lower channel and on the opposite side of the membrane. This arrangement recapitulates the hepatocyte–sinusoidal endothelial interface while also retaining the abundant fenestrations of the liver sinusoid.

Morphology



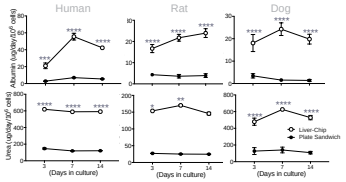
Liver-Chip Morphology
Co-cultured primary hepatocytes and LSECs maintained their stereotypical morphologies in the human, rat, and dog Liver-Chips for up to 14 days in culture.



MRP2 Localization
Liver-Chips showed high transporter expression and localization of MRP2 at the canalicular wall of hepatocytes upon 14 days in culture, whereas plates showed only background staining of non-polarized or dead cells.

Results

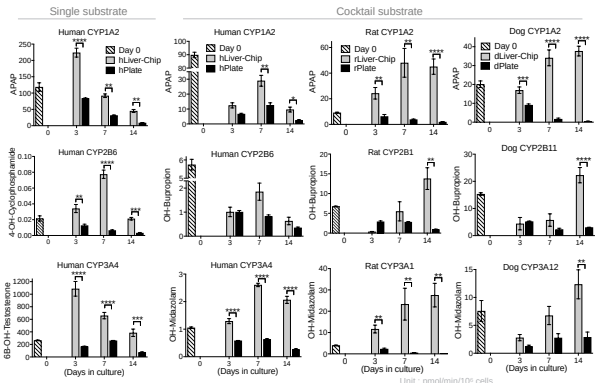
Liver Function



Liver Function
Rates of albumin and urea secretions in human, rat and dog Liver-Chips were evaluated and compared to conventional plate culture over 2 weeks. All three species Liver-Chips maintained albumin and urea secretions during the 2 week time course at robust levels compared to plate sandwich cultured hepatocytes

* p<0.1, ** p<0.01, *** p<0.001, **** p<0.0001

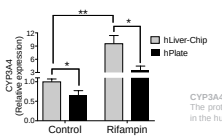
CYP450 Enzyme Activity



Unit : pmol/min/10⁶ cells
* p<0.1, ** p<0.01, *** p<0.001, **** p<0.0001

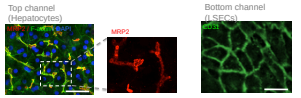
Cytochrome P450 enzyme activity
CYP450 enzyme activity in the Liver-Chips was evaluated and compared to conventional plate culture and day 0 hepatocyte suspension over 2 weeks using single substrate or cocktail probe substrate approaches. Using a single substrate, phenacetin, cyclophosphamide, and testosterone were used to measure human CYP1A2, CYP2B6, and CYP3A4 respectively. Using a cocktail substrate, a mixture of phenacetin, bupropion, and midazolam was used to measure CYP1A, CYP2B, CYP3A respectively in all three species models. Overall, all three species Liver-Chips maintained CYP1A, 2B, and 3A activity during the 2 week time course at robust levels compared to plate sandwich cultured hepatocytes

CYP3A4 Induction



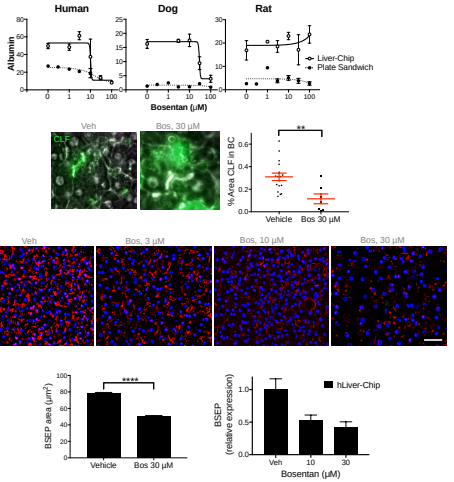
CYP3A4 induction
The prototypical CYP3A4 inducer, rifampin (10 µM), significantly induced CYP3A4 activity in the human Liver-Chip compared to the sandwich culture of hepatocytes in the plate.

Bile Canalicular Network and LSEC Structure



Bile Canalicular Network and LSEC Structure in human Liver-Chip
Confocal images of MRP2 showed branched and elongated bile canalicular networks in hepatocytes. CD31 showed intercellular junctions in LSECs after 14 days in culture.

Bosentan Toxicity in Human, Dog, and Rat Liver-Chips



Bosentan Toxicity in Human, Rat, and Dog Liver-Chips
Bosentan was evaluated in rat, dog, and human Liver-Chips models at concentrations of 1, 10, and 100 µM for 7 days, with daily replacement of treatment media. No treatment-related changes in albumin secretion were detected in the rat Liver-Chip, while albumin secretion was inhibited with an IC_{50} of 29 µM and 12 µM in both the dog and human Liver-Chips, respectively. This indicates a species difference in response to bosentan. The human Liver-Chip was studied further to confirm the hypothesized mechanism of human-specific DILI, which is thought to be mediated by BSEP inhibition, resulting in hepatocellular accumulation of toxic bile acids. The fluorescent hepatic transporter substrates Cholyl-Lysyl-Fluorescein (CLF, BSEP probe) was readily transported across the bile canaliculi in vehicle-treated human Liver-Chip. However, the uptake of the substrate into the canaliculi was significantly inhibited following treatment with 30 µM bosentan, resulting in diffuse cytoplasmic fluorescence that is consistent with the proposed mechanism of toxicity for bosentan. A dose-response of the BSEP inhibition was also demonstrated at concentrations of 3 to 30 µM by specific staining for BSEP and gene expression data.

Conclusions

We report development of Liver-Chip models for three species that showed improved function over conventional monolayer models, as they maintained in vivo-relevant levels of functionality based on albumin and urea secretion, and CYP450 enzyme activity. We evaluated Liver-Chips for each of the three species to assess nonclinical to clinical translation of experimental findings with bosentan at levels that have caused DILI in humans yet were not predicted by animal toxicology studies. In summary, our findings demonstrate the value of multi-species Liver-Chips for predicting human safety testing and risk assessment.

emulate | in collaboration with AstraZeneca | Wyss Institute | Janssen