Comparative Analysis of Species-Specific Hepatocyte Function and Drug Effects in a Liver Microphysiological System PhysioMimix LC12 and 96-Well Plates

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ABSTRACT

Background and Purpose: Drug-induced liver injury (DILI) is an important safety concern in drug development and a common cause of attrition in promising new drug candidates. To help prevent late-stage failure due to DILI, the hepatotoxic potential of a molecule can be assessed using both *in vivo* and *in vitro* models, though these approaches often yield data with uncertain animal-to-human and *in vitro*-to-*in vivo* translatability. Animal-derived *in vitro* liver models can be used to address species-specific differences in adverse liver effects, validate the translatability of *in vitro* tools, and bridge preclinical animal and human findings.

Methods: In this study, we leveraged the PhysioMimix™ LC12 liver platform populated with primary hepatocytes from four main toxicology species (human, monkey, rat, and dog) to identify species-specific differences in DILI. Primary hepatocytes were characterized based on their functional, phenotypic, and transcriptional profiles. Compounds with known species-specific DILI properties including chlorpromazine (CPZ, toxic in all species), bosentan (BOS, toxic in humans), and fialuridine (FIAU, toxic in humans) were compared between 2D (4-day treatment) and 3D (10-day treatment) settings. Liver-specific functional markers (albumin, urea) and injury markers, including lactate dehydrogenase (LDH), were evaluated.

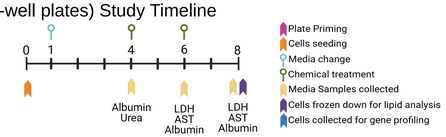
Results: Primary hepatocytes seeded onto the PhysioMimix™ LC12 platform maintained substantially higher, yet gradually declining levels of albumin and urea when compared to their corresponding 2D cultures. LDH remained low in all cultures throughout the experiment. In 2D cultures, CPZ significantly increased LDH release in hepatocytes from all species in a dose-dependent manner. A dose-dependent decrease in albumin secretion was also observed in monkey, rat, and dog hepatocytes following CPZ treatment. No significant changes in urea or other biomarker levels were observed in any test or treatment conditions. By contrast, in the PhysioMimix™ LC12 platform, repeated (10 day) exposure led to a significant increase in LDH release only from human hepatocytes. Additionally, CPZ treatment caused a marked decrease in albumin secretion across all species after 10 days of exposure. Interestingly, only BOS and FIAU reduced albumin secretion, a biomarker of hepatocellular stress in human hepatocytes, with no effect observed in other species.

Conclusions: In conclusion, the PhysioMimix™ LC12 platform supports the physiological function of hepatocytes from different species for up to 14 days. CPZ-, BOS- and FIAU-induced liver toxicity was observed in this model at concentrations ≤100× Cmax. However, high variability and low throughput limited the ability to confidently determine potential species-specific effects of other compounds in the PhysioMimix™ LC12. In 2D cultures, CPZ-induced liver toxicity was also observed, but no significant effects were noted for BOS or FIAU. Ongoing studies are evaluating additional biomarkers, including transcriptional changes following CPZ, BOS, and FIAU treatment.

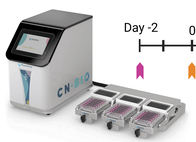
MATERIALS AND METHODS

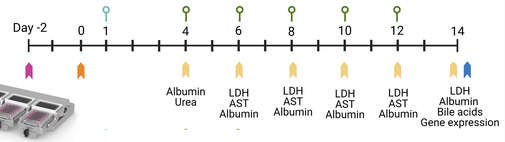
Cell Culture and Drug Exposure (Timepoints)

2D Static culture (96-well plates) Study Timeline



PhysioMimix LC12 MPS Study Timeline





Cell Sourcing

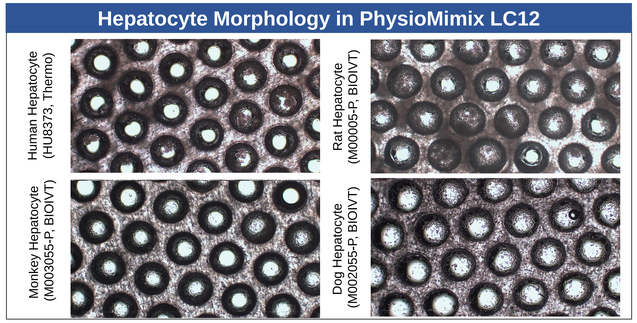
- Human Primary Hepatocytes (HU8373, Thermo)
- Monkey Primary Hepatocytes (M003055-P, BIOIVT)
- Rat Primary Hepatocytes (M00005-P, BIOIVT)
- Dog Primary Hepatocytes (M002055-P, BIOIVT)

Drugs	C_{max}	PhysioMimix LC12	2D Static Cultures
Fialuridine	1 µM	10, 30, 100 µM	1, 10, 30, 100 µM
Bosentan	7.4 µM	10, 30, 200 µM	1, 10, 30, 200 µM
Chlorpromazine	0.5 µM	10, 30 µM	1, 10, 30 µM

Endpoint Assays

- Hepatocyte Function
  - ✓ Albumin
  - ✓ Urea
- DILI Biomarkers
  - ✓ LDH
  - ✓ ALT/AST
  - ✓ Bile acids
  - ✓ Gene expression

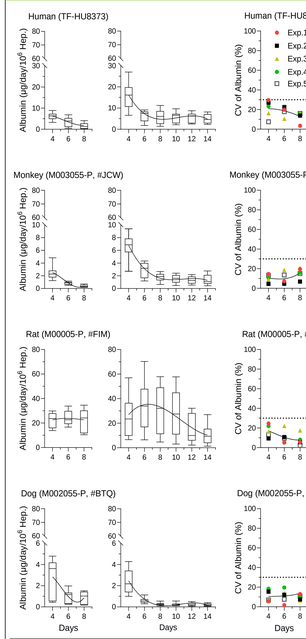
Hepatocyte Morphology in PhysioMimix LC12



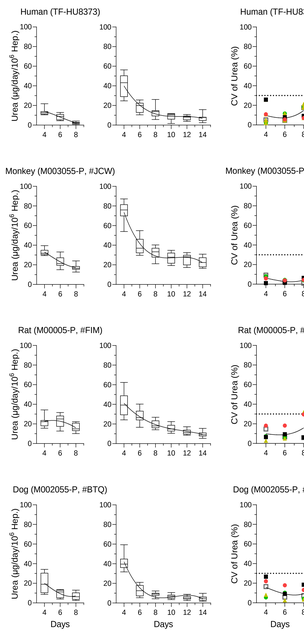
RESULTS

Basal Hepatocyte Function of Human, Monkey, Rat and Dog Cells in 2D or PhysioMimix LC12 Models (14-Day Cultures)

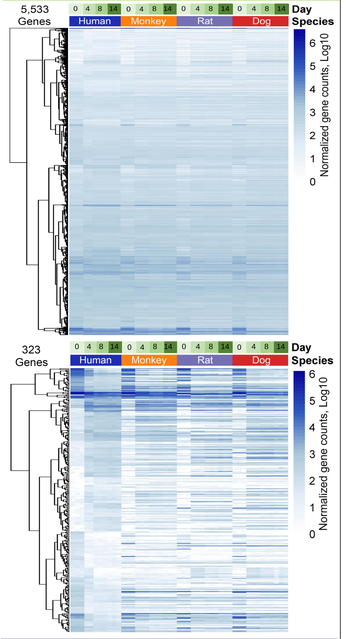
Albumin Secretion & Reproducibility Across Studies



Urea Secretion & Reproducibility Across Studies



Temporal Dynamics in Gene Expression



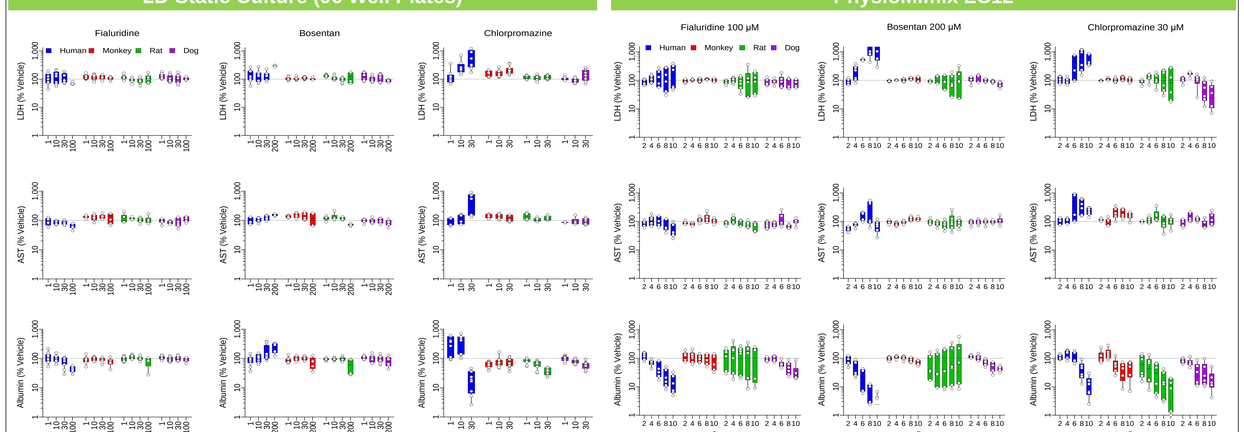
Hepatocytes from human, monkey, rat and dog livers were cultured in 2D (up to 8 days) or the PhysioMimix LC12 MPS (up to 14 days). Over this period, hepatocytes in the MPS demonstrated enhanced function, as evidenced by higher secretion of albumin and urea. This improved secretion profile suggests that the MPS offers a more supportive culture system, promoting the sustained viability and functionality of hepatocytes compared to 2D static culture. Inter-experiment variability analysis showed modest albumin variation (CV ≤30%) in all species except for rat (CV 30-80%). Urea variability was lower (CV ≤20%) across all species. In 2D static 96-well plate experiments, CV ≤20% were observed for both albumin and urea in all species.

Basal gene expression trends in hepatocytes from human, monkey, rat and dog livers that were cultured in PhysioMimix LC12 MPS (up to 14 days). Homologous genes are shown across species. **Top**, all genes; **Bottom**, genes that showed significant differences from Day 0.

Species-Specific Effects of Fialuridine, Bosentan, and Chlorpromazine in 2D and PhysioMimix LC12 Models

2D Static Culture (96 Well Plates)

PhysioMimix LC12



Hepatocytes in the PhysioMimix LC12 showed increased sensitivity towards DILI with prolonged exposures. LDH release was significantly increased in human hepatocytes after prolonged exposure to CPZ, BOS, and FIAU. This increased sensitivity in MPS is likely due to better maintenance of hepatocyte function, and more accurate recapitulation of *in vivo* liver microenvironments. In static 2D cultures, CPZ treatment significantly increases LDH release after short exposure (4 days) in human, monkey, and dog hepatocytes indicating that CPZ induces liver injury across species in this less complex culture system. Similarly, BOS and FIAU-mediated decrease in albumin was observed only in human hepatocytes cultured in PhysioMimix LC12.

SUMMARY

Expected Species-Specific Outcomes

FIAU C_{max} 1 µM	Toxic	Non-toxic	Non-toxic	Non-toxic
BOS C_{max} 7.4 µM	Toxic	Non-toxic	Non-toxic	Non-toxic
CPZ C_{max} 0.5 µM	Toxic	Toxic	Toxic	Toxic

Drug Concentrations Tested per Experiment

	FIAU	BOS	CPZ
Experiment 1	2D: 1- 30 µM MPS: 10 µM	2D: 1- 30 µM MPS: 10 µM	2D: 1- 30 µM MPS: 10 µM
Experiment 2	2D: 1- 30 µM MPS: 30 µM	2D: 1- 30 µM MPS: 30 µM	2D: 1- 30 µM MPS: 30 µM
Experiment 3	2D: 1- 100 µM MPS: 100 µM	2D: 1- 200 µM MPS: 200 µM	2D: 1- 30 µM MPS: 30 µM
Experiment 4	2D: 1- 100 µM MPS: 100 µM	2D: 1- 200 µM MPS: 200 µM	2D: 1- 30 µM MPS: 30 µM

96 Well Plates

	Drug	H	M	R	D
Experiment 1	FIAU	10×	10×	10×	10×
	BOS	1.3×	1.3×	1.3×	1.3×
	CPZ	60×	60×	60×	60×
Experiment 2	FIAU	30×	30×	30×	30×
	BOS	4×	4×	4×	4×
	CPZ	60×	60×	60×	60×
Experiment 3	FIAU	100×	100×	100×	100×
	BOS	30×	30×	30×	30×
	CPZ	60×	60×	60×	60×
Experiment 4	FIAU	100×	100×	100×	100×
	BOS	30×	30×	30×	30×
	CPZ	60×	60×	60×	60×

PhysioMimix LC12

	Drug	H	M	R	D
Experiment 1	FIAU	10×	10×	10×	10×
	BOS	1.3×	1.3×	1.3×	1.3×
	CPZ	20×	20×	20×	20×
Experiment 2	FIAU	30×	30×	30×	30×
	BOS	4×	4×	4×	4×
	CPZ	60×	60×	60×	60×
Experiment 3	FIAU	100×	100×	100×	100×
	BOS	30×	30×	30×	30×
	CPZ	60×	60×	60×	60×
Experiment 4	FIAU	100×	100×	100×	100×
	BOS	30×	30×	30×	30×
	CPZ	60×	60×	60×	60×

2D Static Cultures:

LDH Release (Cytotoxicity Marker):
- CPZ induced a dose-dependent increase in LDH release in human, monkey, and dog hepatocytes.
- BOS increased LDH release only in human hepatocytes.

AST Levels (Hepatocellular Damage Marker):
- AST levels were elevated in human hepatocytes following treatment with CPZ and BOS.

Albumin Secretion (Hepatocyte Function Marker):
- A decrease in albumin release was observed in human and monkey hepatocytes following CPZ treatment.
- FIAU reduced albumin secretion specifically in human hepatocytes.
- No significant effects were observed in other test conditions or species.

PhysioMimix LC12 Cultures with Media Flow:

LDH Release:
- A significant increase in LDH release was observed in human hepatocytes following CPZ, BOS, and FIAU treatment.
- No significant LDH release was detected in hepatocytes from other species.

AST Levels:
- CPZ treatment led to increased AST levels in both human and monkey hepatocytes.
- BOS treatment elevated AST levels in human hepatocytes.

Albumin Secretion:
- CPZ treatment caused a decrease in albumin levels across all species-specific hepatocytes.
- BOS and FIAU reduced albumin secretion only in human hepatocytes.

Overall, our findings suggest that species-specific DILI can be observed in both 2D and MPS cultures at comparable concentrations, 30-100 times in excess of human Cmax. MPS studies were more closely resembling species-specific effects, while 2D experiments showed some false-positive responses.

However, the limitations with throughput in the MPS model precluded dose-finding studies while 2D experiments allowed for more confident determination of the relevant effect levels.

Further studies are needed to test other mechanistic endpoints (such as lipids/bile acids) to determine if they are more sensitive phenotypes.

ACKNOWLEDGEMENTS

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