On the potential of the Human Liver-Chip as a model of cholestatic toxicity

Jake Chaff[1], Heidrun Ellinger-Ziegelbauer[2], Lorna Ewart[1], Stephan Schmidbauer[2], Sushma Jadalannagari[1], James Velez[1], Marian Raschke[3], Thomas Steger-Hartmann[3]

[1]Emulate Inc., 27 Drydock Ave, Boston, MA 02210, USA
[2]Bayer AG, Aprather Weg 18a, 42113 Wuppertal, Germany
[3]Bayer AG, Müllerstrasse 178, 13342 Berlin, Germany

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Background & Purpose

Bile acids are an essential component of bile, which aids digestion in the form of emulsification and absorption of lipids. Primary bile acids (cholic and chenodeoxycholic acid) are synthesized in the liver, can be conjugated with glycine or taurine, concentrated in the gall bladder, and circulated to the intestine during digestion. Primary bile acids are converted to secondary bile acids (deoxycholic and lithocholic acid) by intestinal bacteria and are recirculated back to the liver via enterohepatic circulation.

In a healthy liver, bile acids are secreted from hepatocytes into bile canaliculi through the bile salt export pump (BSEP) which is involved in maintaining bile acid homeostasis. When bile flow is disrupted, increasing levels of intrahepatic bile acids cause cholestatic injury. Cholestasis can be induced by drugs like troglitazone (TROG), a diabetes therapy that was discontinued after causing liver toxicity in clinical trials partly via inhibition of BSEP. **To establish a physiologically relevant model of drug-induced cholestasis, Emulate's human Quad-Culture Liver-Chip was incubated with bile acids in the presence or absence of TROG and monitored for signs of toxicity and BSEP inhibition.** A mixture of glycine-conjugated primary and secondary bile acids, including glycocholic acid (GCA), glycodeoxycholic acid (GDCA) and glycochenodeoxycholic acid (GCDCA), were chosen as they are three of the most abundant bile acids in human serum (comprising ~68% of the total population) and were administered at concentrations ranging from 1-5X human Cmax.

Methods

Primary human hepatocytes were seeded onto an ECM-coated Chip-S1 (Emulate, Inc) while liver non-parenchymal cells (NPCs) were cultured on the opposite side of the membrane. Media was perfused over the Chips, and 6 days post hepatocyte seeding, Chips were pre-treated with GCA, GDCA and GCDCA. Bile acids were administered in ratios corresponding to their human plasma concentrations, ranging from 1X-5X Cmax (250-1250 µM total). After a 2 h bile acid pre-treatment, Chips were treated with 50 µM TROG, with the bile acids present throughout of the study. After 24 and 48 h of treatment, Liver-Chip morphology was assessed, and effluent was collected to measure hepatic function (albumin) and injury (ALT). BSEP function was assessed at 48 h post-treatment by incubating cells with CMFDA, a fluorescently labelled bile acid derivative, and measuring its efflux 0- and 30-min post-incubation.

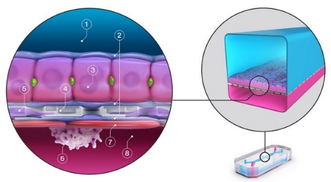


Figure 1 – Human Quad-Culture Liver-Chip overview
Primary human hepatocytes (3) were seeded on a porous, ECM-coated (4) membrane (4) in the top channel (1) of the Chip-S1. A "sandwich culture" was created by overlaying the hepatocytes with Matrigel. Livers NPCs, including liver sinusoidal endothelial cells (LSECs, 7), Kupffer cells (6) and stellate cells (5), were seeded on the opposite side of the membrane in the bottom channel (8), creating a dynamic environment which promotes tissue-tissue interface.

Group	Bile Acid Concentration	TROG Concentration
1	-	-
2		50 µM
3	1X Cmax (250 µM)	-
4		50 µM
5	2X Cmax (500 µM)	-
6		50 µM
7	5X Cmax (1250 µM)	-
8		50 µM

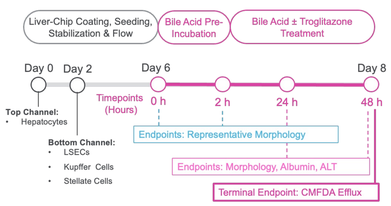
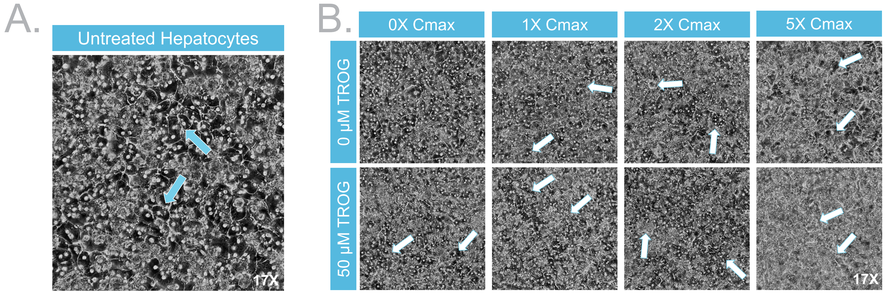


Figure 2 – Experimental parameters to model cholestatic injury
Liver-Chips were pre-incubated ± bile acids, ranging from 1X-5X human Cmax. After a 2 h pre-incubation, Chips were treated ± 50 µM TROG for 48 h to model cholestasis. Toxicity was determined through morphological assessment together with albumin and ALT measurement. BSEP inhibition was measured through CMFDA efflux.

Results

Hepatocytes in the vehicle control group maintained healthy morphology and physiologically relevant levels of albumin (> 20 µg/day/million cells) and ALT release (<10 ng/day/million cells). They further robustly effluxed cleaved CMFDA, suggesting functional BSEP transporters. TROG treatment without bile acid addition caused increased hepatic debris accumulation, a loss of bile canaliculi networks, and a ~50% reduction in albumin secretion after 48 h of treatment. CMFDA efflux was not affected at this timepoint, suggesting BSEP function was not inhibited. Dose-dependent toxicity was observed in response to bile acid treatment with albumin and morphology being the most sensitive indicators of toxicity. 1X Cmax bile acids did not change the morphology relative to the control, but 2X - 5X Cmax bile acids both showed morphological changes such as increased debris accumulation as early as 24 h post-treatment. 1X Cmax bile acids resulted in decreased, but still physiologically relevant levels of albumin, as compared to the vehicle. 2X or 5X Cmax caused toxicity as detected by decreased albumin production. ALT only increased in response to 5X Cmax, showing a spike as early as 24 h post-treatment. Regardless of bile acid concentration, when co-administered with TROG the observed toxicity worsened as measured by morphology and albumin secretion. ALT release was similar between 1X-2X Cmax ± TROG, whereas 5X Cmax + TROG led to the highest ALT release at all timepoints. Qualitatively, when comparing CMFDA levels, it was observed that 1X Cmax + TROG decreased CMFDA efflux which could indicate inhibition of BSEP function. 2X Cmax ± TROG both led to similar levels of CMFDA efflux, which were decreased as compared to the vehicle. 5X Cmax ± TROG led to decreased levels of CMFDA efflux as compared to the control, but 5X Cmax + TROG had the highest levels of internalized CMFDA, suggesting the most inhibition of BSEP.



C.

Treatment	Top Channel - Hepatocytes			Bottom Channel - NPCs		
	0 h	24 h	48 h	0 h	24 h	48 h
VEH	0	0	0	0	0	0
TROG	0	0	1	0	0	0
1X Cmax	0	0	1	0	0	0
1X Cmax + TROG	0	0	1.5	0	0	0
2X Cmax	0	1	1.5	0	0	0
2X Cmax + TROG	0	2	2	0	0	0
5X Cmax	0	2	3	0	0	0
5X Cmax + TROG	0	2.5	4	0	0	0

Healthy 0 1 2 3 4 Damaged
0 1 2 3 4

Figure 3 – Liver-Chip morphology and toxicity assessment
(A) Pre-treatment, the hepatocytes demonstrated healthy morphology. A healthy hepatocyte is defined as having cuboidal, often binucleated morphology, with clear, in-tact bile canaliculi. The NPCs (not pictured) also demonstrated healthy morphology with a confluent monolayer. (B) After 48 h the vehicle (VEH) demonstrated healthy morphology, whereas a dose-dependent toxicity was observed in response to the bile acids treatment. Toxicity was worsened wherever TROG was applied, suggesting possible cholestatic injury. White arrows denote areas where toxicity is present. Toxicity was observed in the form of debris accumulation, loss of bile canaliculi networks, and peeling from the Chip. (C) Hepatocytes and NPCs were scored based on apparent toxicity, with a score of 0 denoting health, and a score of 4 denoting severe toxicity. The hepatocytes demonstrated a dose- and time-dependent toxicity in response to the bile acid treatment. TROG treatment worsened the apparent toxicity when applied. The NPCs were untreated and demonstrated similar healthy morphology regardless of hepatic treatment.

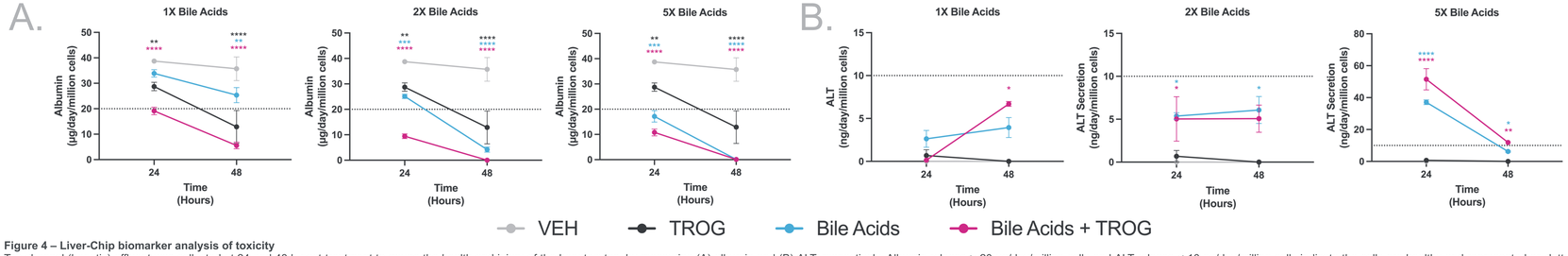


Figure 4 – Liver-Chip biomarker analysis of toxicity
Top channel (hepatic) effluent was collected at 24 and 48 h post-treatment to gauge the health and injury of the hepatocytes, by measuring (A) albumin and (B) ALT, respectively. Albumin release > 20 µg/day/million cells and ALT release < 10 ng/day/million cells indicate the cells are healthy and represented as dotted lines on the graphs above. The untreated control Chips (VEH) demonstrated physiologically relevant levels of albumin release, and low levels of ALT release through 48 h of monitoring, suggesting the untreated Chips were healthy. TROG treatment led to decreased albumin production, but no relevant spikes in ALT were observed, suggesting the onset of toxicity. 1X bile acids alone led to decreased albumin and a slight increase in ALT as compared to the VEH, but both values were within an acceptable range, so toxicity was negligible. 1X bile acids + TROG led to a statistically significant and biologically relevant decrease in albumin, suggesting toxicity. A small increase in ALT was also observed in response to this treatment.

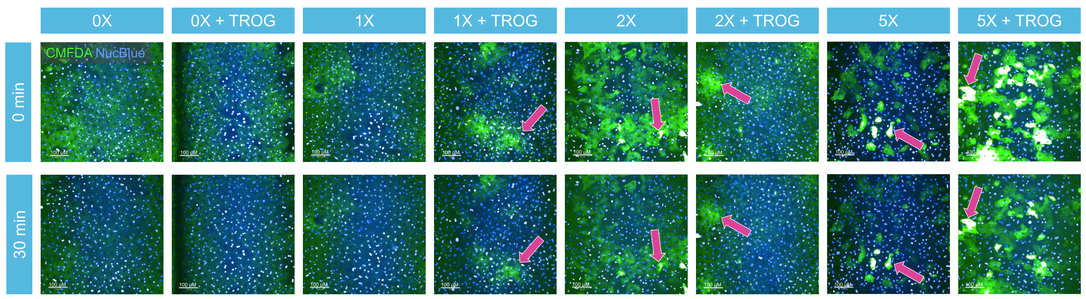


Figure 5 – BSEP inhibition as measured through CMFDA Efflux
CMFDA is a bile acid derivative which passively enters cells and is cleaved to become a cell-impermeant fluorescent molecule. Once it is transformed inside hepatocytes, it can only exit through BSEP. Hepatocytes were incubated with CMFDA for 30 min, and then were imaged at 0- and 30-min post-incubation to gauge efflux capabilities.

Qualitatively, the untreated control was able to almost completely efflux CMFDA after 30 min of monitoring. TROG similarly was able to robustly efflux CMFDA as well. These results suggest BSEP function was not impaired in response to 0X Cmax bile acids ± TROG. 1X Cmax bile acids was able to efflux CMFDA like the control, whereas 1X Cmax bile acids + TROG led to decreased efflux efficiency, suggesting the first signs of BSEP inhibition (pink arrows). 2X ± TROG showed a similar decreased efflux efficiency. 5X ± TROG showed a near complete inhibition of CMFDA efflux, with 5X + TROG demonstrating the highest levels of internalized CMFDA across the board.

Conclusion

Cholestasis is one of the most common forms of DILI, thus the capabilities of the Liver-Chip were explored to see if it could be a model for cholestatic-like toxicity. Results demonstrated a time- and dose-dependent toxicity in response to bile acid treatment. The addition of TROG worsened toxicity, primarily via albumin release, morphology, and inhibition of CMFDA efflux. This allows for future applications of comparing and predicting the toxicity of other cholestatic-inducing drugs or developing therapies to better remedy cholestasis.