Predicting Hepatotoxicity: Integration of Transport, Metabolism and Bile Acid Regulation

Christopher B. Black, Jonathan P. Jackson, Matthew K. Palmer, Caroline L. Moseley, and Kenneth R. Brouwer
BioIVT, ADME-TOX, 2810 Meridian Parkway, Suite 100, Durham, NC 27713

INTRODUCTION

Bile acid homeostasis is critical in assessing cholestatic liver injury in humans and has been associated with bile salt export pump (BSEP) inhibition (IC_{50}s < 25 µM)[1]; however, *in vitro* BSEP IC_{50} concentrations do not correlate with *in vivo* cholestatic DILI severity. Sandwich-cultured human hepatocytes (SCHH), when treated with BSEP inhibitors, respond to the resulting increased intracellular concentrations of bile acids (BA), via activation of FXR (adaptive response). This results in decreased synthesis of BA and increased expression of basolateral and canalicular efflux of BA via OSTα/β, and BSEP which prevents cholestatic hepatotoxicity. We propose that BSEP inhibition alone may not be sufficient to induce toxicity due to this adaptive response. In addition to BSEP inhibition, inhibition of basolateral efflux and/or interference with the adaptive response (FXR antagonism) may lead to increases in drug-induced cholestatic bile acid hepatotoxicity. Such mechanisms must be incorporated to accurately predict *in-vivo* cholestatic drug induced liver injury (DILI).

Hepatotoxicity can result from direct effects of a drug, or from a failure to adapt to internal processes altered by drug administration. A predictive model for bile acid homeostasis and cholestatic hepatotoxicity (C-DILI™ Assay) was developed which integrates direct and adaptive effects of a drug, including: BSEP inhibition, basolateral efflux inhibition, and FXR antagonism using a human hepatocyte system.

METHODS

Human Hepatocytes Cryopreserved, Transporter Certified™ human hepatocytes in a sandwich configuration were cultured using QualGro ™ Media for 5 days.

Treatments On Day 4 of culture, hepatocyte cultures were exposed to test compounds (Table 1) at 20X (or limit of solubility) of their systemic C_{max} in Qual-Gro™ Sensitization Media (physiologically relevant concentrations of lipids and bile acids) for 24 hours. Approximately 49 compounds with a range of BSEP IC_{50}s and associated with clinical DILI were tested. Treatment with vehicle control and standard QualGro™ Media was used as a control.

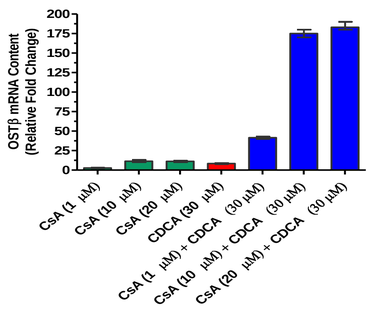
Gene Expression mRNA content of various transporters, synthetic enzymes, and regulatory factors from SCHH was determined from each RT reaction using gene-specific TaqMan® primer/probe sets. All reactions were normalized to the endogenous control GAPDH. Amplifications were performed on an ABI ViiA7 Real-Time PCR System in relative quantification mode. Relative-fold mRNA content was determined for each treatment group relative to the vehicle control.

Endogenous Bile Acids LC-MS/MS which employed reversed-phase HPLC and electrospray ionization was used to quantitate endogenously generated cholic acid (CA), CDCA, and their taurine (TCA, TCDCA) and glycine (GCA, GCDCA) conjugates in cells, bile and cell culture media.

Figure 2 Increased OSTβ expression through activation of FXR (adaptive response) observed:

- in the presence of a BSEP inhibitor (CsA)
- Increased intracellular concentration of bile acids by addition of CDCA

Synergistic effect observed in the presence of CsA **and** CDCA due to greatly increased intracellular concentrations of bile acids



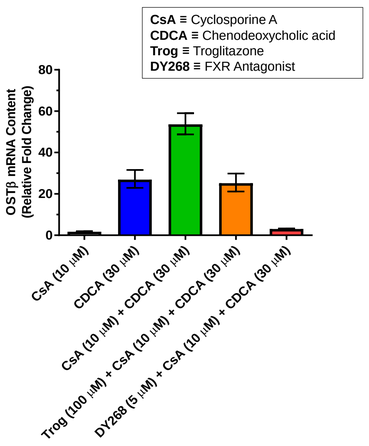
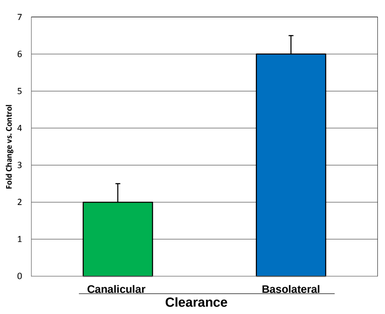


Figure 3 Decrease in OSTβ mRNA expression when troglitazone (weak FXR antagonist) or DY268 (strong FXR antagonist) are co-administered with CsA and CDCA

FXR antagonism can prevent the hepatocyte from responding to high intracellular concentrations of bile acids and increasing the potential for cholestatic hepatotoxicity

Increase in Canalicular and Basolateral Efflux Clearance Following Exposure to CDCA



Guo, et.al. J Pharmacol Exp Ther 365:413–421, May 2018

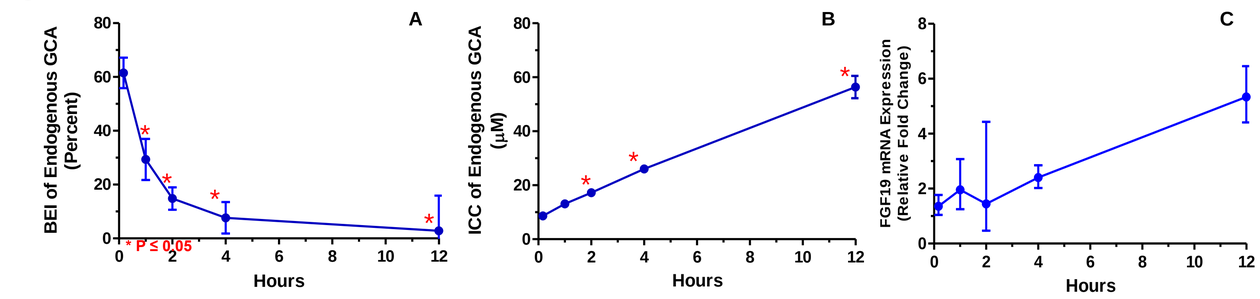
Figure 4 Chronic exposure to CDCA increases mRNA expression of BSEP (canalicular) and OSTα/β (basolateral) efflux by approximately 8X and 80X, respectively[2]

Molecular modeling was utilized to estimate changes in the canalicular and basolateral efflux clearances following 72 hours of exposure to CDCA (100 µM). Canalicular clearance increased by 2X while the basolateral clearance increased by 6X.

In the induced state basolateral efflux clearance mediated by OSTα/β represents an important elimination pathway.

RESULTS AND DISCUSSION

Figure 1



Exposure to Cyclosporine A (10 µM), a potent BSEP inhibitor leads to a **rapid, time dependent decrease** in biliary excretion of endogenous bile acids.

Inhibition of biliary excretion leads to an increase in the intracellular concentration of endogenous bile acids.

Increased intracellular concentrations of bile acids activate FXR (increased FGF19).

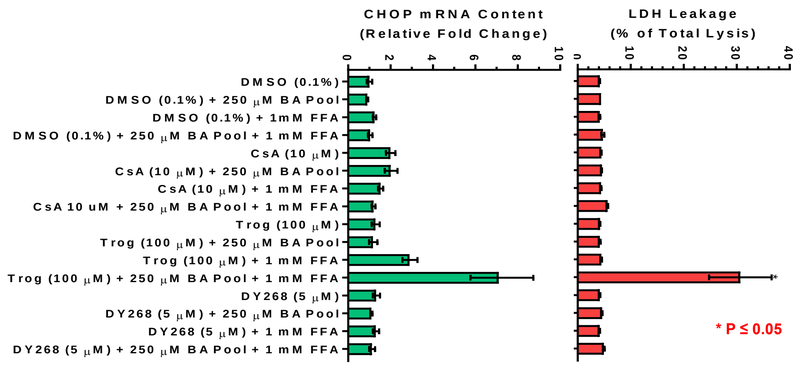


Figure 5 CCAAT/enhancer-binding protein homologous protein (CHOP) is a key component in the ER stress-mediated cell death pathways. CHOP mRNA expression was increased > 3-fold in SCHH treated with troglitazone under sensitization conditions (e.g. BA pool + FFA) for 12 hours. Concomitant increase in LDH leakage > 7.3-fold above solvent control was also observed. These results suggested troglitazone treatment under sensitization conditions induced ER stress resulting in BA-induced (e.g. cholestatic) hepatocyte injury.

C-DILI™ Assay

The cultures were measured for ATP content (CellTiter-Glo® Promega) and LDH secretion (CytoTox-ONE™ Promega) following 24 hours of treatment. The LiverTox data base was used to identify compounds that were consistent with hepatocellular injury. LDH readout was used as a surrogate marker of cholestatic bile acid toxicity.

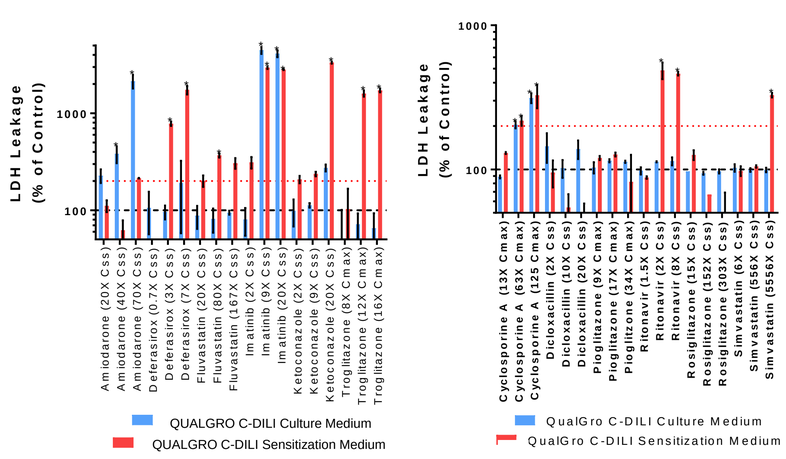


Figure 6 A selection of compounds were re-evaluated across a range of concentrations in a separate lot of hepatocytes to evaluate dose-dependency and to confirm previous results. * p-value ≤ 0.05

Table 1 49 compounds with varying degrees of BSEP inhibition and clinical DILI (NIH LiverTox database) were evaluated in the C-DILI™ Assay. The C-DILI™ Assay reduces the number of false positives predicted by hBSEP IC_{50} cutoff of 25 µM and identifies mode of toxicity. NA-not applicable.

Drug	hBSEP Vesicle IC_{50} (µM)[3]	C-DILI™ Assay Hepatotoxicity Potential	C-DILI™ Assay Hepatotoxicity Mechanism
Acitretin	38	Low Potential	NA
Amiodarone	43	High Potential	General
Atorvastatin	13	Low Potential	NA
Bosentan	23	Low Potential	NA
Calcitrol	40	Low Potential	NA
CsA	0.5	Low Potential	NA
Clofibrate	71	Low Potential	NA
Deferasirox	58	High Potential	Cholestatic
Dicloxacillin	56	Low Potential	NA
Dipyidamole	3.8	Low Potential	NA
Donepezil	78	Low Potential	NA
Entacapone	56	Low Potential	NA
Eryth. Estolate	13	Low Potential	NA
Everolimus	2.0	Low Potential	NA
Ezetimibe	56	Low Potential	NA
Febuxostat	43	Low Potential	NA
Flupirtine	36	Low Potential	NA
Fluvastatin	36	High Potential	Cholestatic
Glyburide	5.0	Low Potential	NA
Iloperidone	23	Low Potential	NA
Imatinib	25	High Potential	General
Indomethacin	42	Low Potential	NA
Irbesartan	7.3	Low Potential	NA
Ketoconazole	3.4	High Potential	Cholestatic
Lapatinib	6.5	Low Potential	NA
Losartan	8.5	Low Potential	NA
Megestrol Acetate	18	Low Potential	NA
Mifepristone	2.0	Low Potential	NA
Nicardipine	7.9	Low Potential	NA
Nifedipine	64	Low Potential	NA
Pazopanib	10	Low Potential	NA
Pioglitazone	0.3	Low Potential	NA
Posaconazole	8.1	High Potential	General
Pranlukast	2.9	Low Potential	NA
Primaquine	33	Low Potential	NA
Repaglinide	22	Low Potential	NA
Reserpine	8.4	Low Potential	NA
Rifabutin	27	Low Potential	NA
Ritonavir	1.6	High Potential	Cholestatic
Rosiglitazone	2.8	Low Potential	NA
Simvastatin	25	Low Potential	NA
Sitaxsentan	13	Low Potential	NA
Tacrolimus	7.2	Low Potential	NA
Telithromycin	5.0	Low Potential	NA
Telmisartan	16	Low Potential	NA
Tolcapone	37	High Potential	General
Tolvaptan	9.9	Low Potential	NA
Troglitazone	2.7	High Potential	Cholestatic
Zafirlukast	11	Low Potential	NA

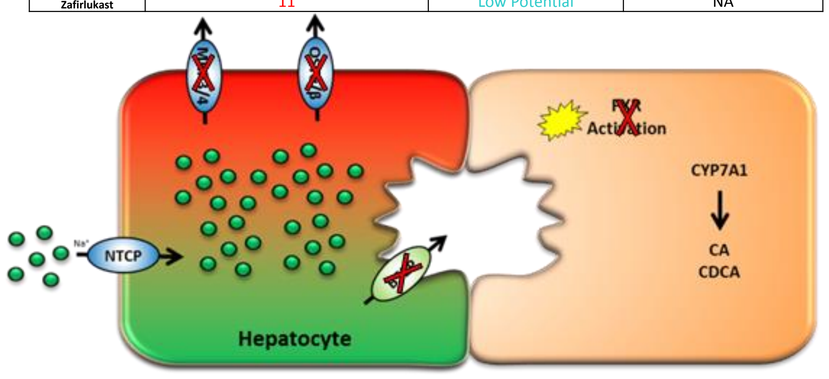


Figure 7 Inhibition of BSEP along with antagonism of FXR activation in response to increased intracellular concentrations of bile acids, and/or inhibition of bile acid basolateral efflux transporters can lead to increased cholestatic hepatotoxicity.

Drugs that impact multiple pathways or single pathways when given in combination with other drugs that affect alternate pathways could also result in hepatotoxicity. E.g. drug interaction with a BSEP inhibitor and FXR antagonist or basolateral efflux transporter inhibitor.

CONCLUSIONS

The C-DILI™ Assay correctly predicted compounds with significant clinical hepatocellular cholestatic toxicity by:

- Integrating effects on BSEP, OSTα/β, MRP3/4, and FXR to delineate hepatotoxicity resulting from a build up of intracellular bile acids.
- Taking into account a compound's effect on the regulation of bile acid homeostasis, an important pathway for hepatotoxicity.

Key Assay Features:

- Transporter Certified™ human hepatocytes in a model that maintains transporter expression, localization and function
- Optimized culture media
- Convenient 24 hour incubation in 96-well format
- Assess effects of parent and metabolites simultaneously
- Integrated effects of transporter inhibition (acute effects) and regulation (chronic effects) in a single toxicity readout
- Evaluate cholestatic vs. direct toxicity
- Available as a service or kit

[1] Dawson et.al., Drug Metab Dispos 40,130, 2012, [2] Jackson et.al., App In Vitro Tox 2, 4, 2016, [3] Morgan et.al. Tox Sci 136,1, 2013, [4] https://livertox.nih.gov/
[5] Malinen et.al. Am J Physio – GI & Liver Physio. Feb 2018

Contact information: cblack@bioivt.com