A New Mechanism-Based Human *In Vitro* Screen (C-DILI™ Assay) that Accurately Predicts Cholestatic Liver Toxicity

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INTRODUCTION

Cholestatic DILI in humans 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 basolateral efflux of BA via OSTα/β, which prevents cholestatic hepatotoxicity. Therefore, BSEP inhibition alone may not be sufficient to induce toxicity. 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).

The C-DILI™ Assay is a novel predictive model for cholestatic hepatotoxicity that integrates the effects of BSEP inhibition, basolateral efflux inhibition, and FXR antagonism using a human hepatocyte system that recapitulates clinically relevant intracellular concentrations.

METHODS

Human Hepatocytes Cryopreserved, Transporter Certified™ human hepatocytes in a sandwich configuration (96-well format) were cultured using QualGro ™ Media for 5 days., hepatocytes were exposed to test compound .

Treatments On Day 5 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 50 compounds with a range of BSEP IC_{50}s and clinical cholestasis were tested. Treatment with DMSO (0.1%) and standard QualGro™ Media was used as a control.

Analytical 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.

[1]Dawson et al., Drug Metab Dispos 40:130, 2012

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Table 1. Selected Compounds

Compound	Test Concentration (uM)
Amiodarone	30.0
Atorvvastatin	0.25
Bosentan	90.0
Clofibrate	500
Cyclosporine A	10.0
Deferasirox	400
Dicloxacillin	300
Dipyidamole	23.0
DMSO	0.10%
Donepezil	8.00
Entacapone	35.0
Erythromycin Estolate	84.0
Everolimus	0.50
Ezetimibe	3.00
Febuxostat	21.0
Flupirtine	17.0
Fluvastatin	1.20
Iloperidone	0.18
Imatinib	88.0
Indomethacin	22.0
Ketoconazole	26.0
Megestrol	30.0
Mifepristone	75.0
Nifedipine	3.00
Pioglitazone	58.0
Pranlukast	8.00
Primaquine	1.50
Repaglinide	0.33
Rifabutin	0.02
Ritonavir	350
Rosiglitazone	7.00
Simvastatin	0.20
Sitaxsentan	60.0
Solithromycin	52.0
Tacrolimus	0.25
Telithromycin	20.0
Telmisartan	3.00
Tolvaptan	8.00
Troglitazone	100

Table 2 (right). Correct identification of the specific type of liver injury is critical for correlation with clinical outcomes. Bile Duct Injury is typically associated with immune reactivity and cell damage to cholangiocytes.

https://livertox.nih.gov/

RESULTS AND DISCUSSION

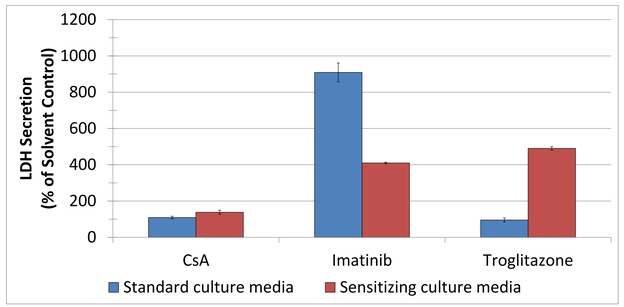

Figure 1. Direct Toxicity vs. Cholestatic Toxicity: Media composition is critical. Cyclosporine A (CsA), a potent BSEP inhibitor, was not toxic in either media. Imatinib treatment caused toxicity in both media types suggesting that toxicity was direct. Troglitazone caused toxicity only on cells with sensitization media indicating compound disrupts bile acid homeostasis.

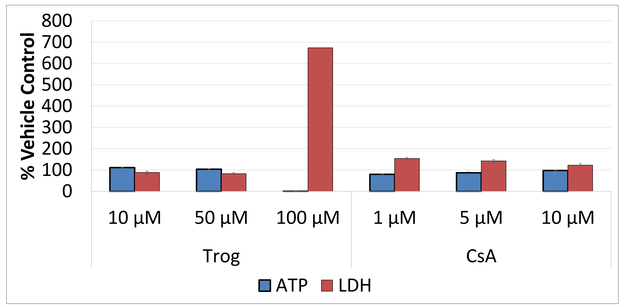

Figure 2. In the C-DILI™ Assay, CsA does not cause toxicity. Toxicity is only observed when compounds impact multiple pathways, e.g. Troglitazone (and metabolites) inhibit BSEP and are FXR antagonists.

DILI Liver Injury Patterns		
	Hepatocellular Injury	Bile Duct Injury
Description	Hepatitis-like injury	Bile duct flow blockage
Manifests As	marked liver cell necrosis	portal inflammation, bile duct injury
Clinical Symptoms	fatigue and weakness	jaundice and increased itching
AP and GGT	↑	↑↑↑
ALT and AST	↑↑↑	↑
Bilirubin	↑	↑↑↑

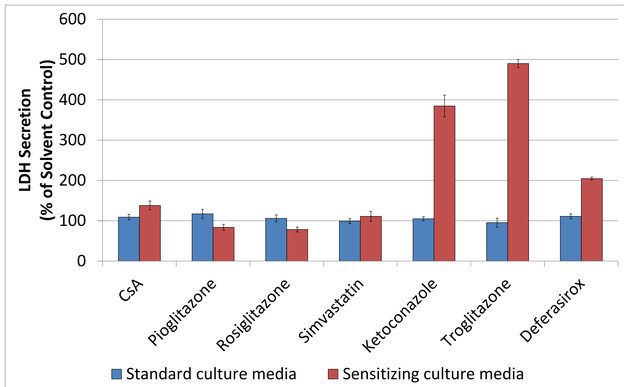

Figure 3. CsA, Pioglitazone, Rosiglitazone, Simvastatin were not toxic in either media. Ketoconazole and Troglitazone, and Deferasirox caused toxicity in sensitizing culture media only. These results indicated that Ketoconazole, Troglitazone, and Deferasirox disrupt bile acid homeostasis resulting in cholestatic hepatotoxicity

Compound	hBSEP IC50 (µM)	hBSEP Result (≤25 µM)	C-DILI™ Assay Result	Clinical Liver Injury (Incidence)
Cyclosporin A	0.5	High Potential	Low Potential	None or rare
Pioglitazone	0.5	High Potential	Low Potential	None or rare
Rosiglitazone	3	High Potential	Low Potential	None or rare
Simvastatin	25	High Potential	Low Potential	None or rare
Ketoconazole	3	High Potential	High Potential	Hepatocellular (1:2000)
Troglitazone	3	High Potential	High Potential	Hepatocellular (1:1000)
Imatinib	25	High Potential	Direct Toxicity	Mixed
Deferasirox	58	Low Potential	High Potential	Hepatocellular

Table 3. The C-DILI™ Assay correctly predicted hBSEP False Positive (Rosiglitazone) as having low potential for hepatocellular cholestasis, and hBSEP False Negative (Deferasirox) as having a high potential. Troglitazone and Ketoconazole, both True Positives in the hBSEP analysis were also correctly predicted in the C-DILI™ Assay.

CONCLUSIONS

- **The C-DILI™ Assay Correctly predicted compounds with significant clinical hepatocellular cholestatic toxicity**
  - Integrates effects on BSEP, OST, MRP3/4, and FXR to delineate hepatotoxicity resulting from a build up of intracellular bile acids
- **Key Assay Features:**
  - Transporter inhibition and regulation in a single toxicity readout
  - 24 hour incubation in 96-well format
  - Assess effects of parent and metabolites
  - Assess cholestatic vs. direct toxicity