Cholestatic DILI in humans has been associated with bile acid export pump (BSEP) inhibition; however, in vitro BSEP C50 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 concentration (ICC) of bile acids (BA), by activation of FXR (adaptive response). This results in decreased synthesis of BA and increased expression of basolateral and canalicular efflux transporters for BA via OATP alpha/beta, and BSEP which prevents cholestatic hepatotoxicity. We evaluated the time course of this adaptive response, changes in the ICC of BA, the effects of FXR antagonists, and the in vivo relevance, and whether interaction of FXR regulatory effects would improve the prediction of cholestatic DILI. Cryopreserved, TRANSPORTER CERTIFIED™ human hepatocytes were maintained in a sandwich configuration were cultured using QUALGRO™ Media for 5 days. On Day 5 of culture, the time course of the adaptive response was determined by determining the effect of cyclosporine A on the biliary excretion, and ICC of endogenous bile acids (LCMS analysis), in parallel with FXR activation (gene expression - TaqMan® primer/probe sets). Mechanistic modeling was used to determine the functional effects of mRNA based changes in FXR activation. The effect of the ER stress biomarker, CHOP, following 12 hours of exposure to CsA (10 µM), Troglitazone (100 µM), or Dy268 (5 µM) under sensitization conditions (250 µM BA pool + 1 mM free fatty acids (FFA)) was also evaluated. In a separate study, 49 compounds with varying degrees of BSEP inhibition and DILI (NHL liverTox database) were evaluated (24 hr exposure, sensitization resulted in a 2X increase in the biliary efflux clearance, and a 6X increase in the basolateral efflux clearance (adaptive response). Co-administration of FXR antagonists reduced the FXR mediated response to 50% of control for troglitazone and Dy268, respectively. Following 12 hours of exposure, CHOP mRNA content was induced ≥2.0-fold above solvent control in SCHH treated with Troglitazone (100 µM) in the presence of BA pool + FFA. Integration of the effect on the adaptive response in addition to the effect on BSEP inhibition improved the accuracy for prediction of cholestatic DILI from 22% (BSEP inhibition alone) to 93%. In addition to BSEP inhibition, integration of inhibition of basolateral efflux and/or interference with the adaptive response (FXR antagonism) allows for more accurate prediction of cholestatic DILI.

**O B J E C T I V E S**

**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 Cmax in QUALGRO™ Sensitization Media (physiologically relevant concentrations of lipids and bile acids) for 24 hours. Approximately 49 compounds with a range of BSEP IC50s 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 mRNA content was determined for each treatment group relative to the vehicle control.

**Key assay features:**

- TRANSPORTER CERTIFIED™ human hepatocytes in a sandwich configuration
- Cryopreserved
- Basolateral efflux is an important BA efflux pathway following FXR activation

**RESULTS AND DISCUSSION**

**CONCLUSIONS**

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

- Integrates effects on BSEP, OATP/B, MRP3/4, and FXR to delineate hepatotoxicity resulting from a build up of intracellular bile acids
- BSEP inhibition alone does not result in BA-induced (e.g. cholestatic) liver injury
- Contingency Analysis: 95% Accuracy, 81% Sensitivity Score (ability to predict toxicity), 100% Specificity Score (ability to predict no toxicity)

**C-DILI™ Assay results consistent with clinical evidence of liver injury**

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-DILI™ Assay</th>
<th>C-DILI™ Assay</th>
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<th>C-DILI™ Assay</th>
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</thead>
<tbody>
<tr>
<td>Troglitazone</td>
<td>2X Cmax</td>
<td>20X Cmax</td>
<td>≥2.0-fold</td>
<td>≥2.0-fold</td>
</tr>
</tbody>
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**Bile acid transporters**

- BSEP
- MRP3/4
- FXR

DILI (NIH LiverTox database) were evaluated in the C-DILI™ Assay.

**Figure 4**

- CCAAT/enhancer binding homogenous protein (CHOP) is a key component of the ER stress-mediated cell death pathways. CHOP mRNA expression was increased > 3-fold in SCHH treated with troglitazone under sensitization conditions (100 µM BA pool + FFA) for 12 hours. Concomitant increase in LDH leakage > 2.5-fold above solvent control also was observed. These results suggested troglitazone treatment under sensitization conditions induced ER stress resulting in BA-induced (e.g. cholestatic) hepatocyte injury.

**Figure 5**

- 47 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 BSEP C50 cutoff of 25 µM and identifies mode of toxicity. NA not applicable.

**Contact information**

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