BSEP inhibition is a “triggering” event that leads to the indirect activation of FXR via increased intracellular concentrations of bile acids (BA). Activation of FXR initiates a basolateral efflux compensatory mechanism via OSTA/β that reduces intracellular concentration of BA preventing hepatotoxicity. This mechanism explains the weak concordance between BSEP inhibition potency and DILI incidence and is consistent with clinical observations showing that increases in circulating BA are not always associated with drug induced liver injury (DILI). Under this new paradigm, compounds must inhibit bile acid efflux in addition to blocking FXR activation through antagonism or inhibiting basolateral BA efflux via OSTA/β to cause cholestatic DILI. To prospectively evaluate a new chemical entity’s (NCE) cholestatic DILI potential, an appropriate model system must support the necessary biological processes involved in BA homeostasis, while supporting nuclear receptor signaling. Using TransPorter Certified™ sandwich-cultured human hepatocytes (SCHH), the C-DILI™ Assay (patent pending) is a novel predictive cholestatic hepatotoxicity model that evaluates a NCE’s potential to disrupt BA homeostasis mechanism (e.g. BSEP inhibition, basolateral efflux inhibition, and FXR antagonism). Utilizing this technology, we evaluated the thiazolidinedione class of compounds including troglitazone, pioglitazone, and rosiglitazone to determine the feasibility of using the C-DILI™ assay and mechanistic studies (e.g. BSEP inhibition, FRX antagonism) in SCHH to develop structural activity relationships (SAR) within a compound class.

The expression of FGF19, a sentinel marker of FXR activation, was evaluated in SCHH following 24 hours treatment. A) The BSEP inhibitor CA5 (10 μM increased FGF19 mRNA content 1.9-fold above solvent control. The FXR agonist CDCA (30 μM) increased FGF19 mRNA 23.3-fold above solvent control. A synergistic increase in FGF19 mRNA content of 62.8-fold was observed in SCHH co-treated with CA5 (10 μM) and CDCA (30 μM). Values represent the mean and error bars represent 95% confidence intervals.

Following exposure to CA5 or thiazolidinediones, the bile clearance of the model bile acid dB-TCA was evaluated in SCHH utilizing B-CLEAR™ technology. Biliary clearance of dB-TCA was reduced for approximately 40% of solvent control in SCHH co-treated with CA5 or troglitazone. The BEL, a measure of biliary efflux, of dB-TCA was reduced in a dose-dependent manner in SCHH treated with CA5 (1-100 μM) or Troglitazone (100 μM). Reduction of dB-TCA bile efflux was also demonstrated by biliary partition factor, a ratio of dB-TCA bile accumulation to dB-TCA cellular accumulation. Mean and SD of triplicate wells are reported.

Materials and Methods

Preparation of sandwich-culture hepatocytes: Sandwich cultured human Hepatocytes (SCHH) were established with TransPorter Certified™ cryopreserved cells for use in 48-well hepatobiliary disposition (Figure 2), gene expression (Figure 3) and toxicology studies (Figure 6). Once thawed the cells were suspended in QualGro™ Seeding Medium, a QG proprietary medium, at a density of 0.8-1.0 million viable cells/ml and seeded onto BioCoat™ 38-well cell culture plates purchased from Corning. Following the initial seeding, cells were allowed to attach for 2.5 hours, then rinsed and fed with 200 μl/well warm (37°C) QualGro™ Seeding Medium. After a culture time of 18-24 hours, the seeding medium was removed and the cells were fed and overlaid with QualGro™ Culture Medium supplemented with 30 μg/mL Mitomycin C. Cells were maintained in QualGro™ Culture Medium, a QG proprietary medium, supplemented with 30 μg/mL Mitomycin C, for up to 14 days. Biliary Cells were maintained in QualGro™ Culture Induction Medium until consumed. All hepatocyte were consumed on day 5 of culture.

C-DILI™ Assay (Figure 1): SCHH were established by thawing TransPorter Certified™ cryopreserved cells according the manufacturer’s instructions. Once thawed the cells were suspended in QualGro™ Seeding Medium at a density of 0.8 million viable cells/ml, and seeded onto BioCoat™ 38-well cell culture plates purchased from Corning. Following the initial seeding, cells were allowed to attach for 2.5 hours, then rinsed and fed with 200 μl/well warm (37°C) QualGro™ Seeding Medium. After a culture time of 18-24 hours, the seeding medium was removed and the cells were fed and overlaid with QualGro™ Culture Medium supplemented with 30 μg/mL Mitomycin C. Cells were maintained in QualGro™ Culture Medium, a QG proprietary medium, supplemented with 30 μg/mL Mitomycin C, for up to 14 days. Biliary Cells were maintained in QualGro™ Culture Induction Medium until consumed. All hepatocyte were consumed on day 5 of culture.

Bile Acid Disposition Assay (Figure 2): To investigate the biliary and intracellular disposition of BA into SCHH bile pockets, SCHH were established as described above. On day 5 of culture, SCHH were exposed to varying concentrations (5-50 μM) of troglitazone (100 μM for 24 hours). Following the incubation, the wash solution was removed and replaced with fresh PBS (5 ml) buffer. The plates were then flushed with 37°C pre-warmed medium until all of the fluorescent content was eluted. Total fluorescence was measured using a Tecan Infinite M200. Bile acid content was determined using a validated HPLC assay. Total bile acid content was determined using a validated HPLC assay.

Total RNA isolation and qRT-PCR (Figure 3): Total RNA isolation and qRT-PCR were performed as previously described.

References

2. Liver Toxicity Database. Clinical and Research Information on Drug-induced Liver Injury Database. https://www.liver.tox.com/about.html