Effect of Drugs that Cause Cholestatic Drug-Induced Liver Injury on Bile Acid Transport and Metabolism

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Background

Drug-induced liver injury (DILI) is the leading cause of acute liver failure and a major reason for liver transplantation [1]. One of the most prominent forms of clinically observed DILI is cholestatic DILI [2]. Cholestasis is characterised by impaired hepatic bile flow and may be induced by several different mechanisms. Cholestatic DILI can occur as the sole DILI phenotype or as a comorbidity with other DILI phenotypes.

In order to reduce the occurrence of cholestatic DILI and other adverse drug reactions, it is vital to develop the tools and knowledge to comprehensively screen drugs for toxicity. C-DILI™ and B-CLEAR® assays can be used to screen drugs for cholestatic DILI potential and the capacity to impair biliary transport, respectively.

Methods

Cell Culture: Transporter Certified™ cryopreserved primary human hepatocytes (PHH) were cultured in a sandwich configuration. HuH-7 cells were cultured as described previously [3].

C-DILI™ Assay: PHH (n=3) were exposed to fialuridine, pioglitazone, ritonavir and troglitazone for 24 h in either C-DILI™ culture media or sensitization media at five concentrations, covering the ranges listed in the table:

Drug	Concentration Range (µM)
Fialuridine	15.6 – 250
Pioglitazone	31.3 – 500
Ritonavir	15.6 – 250
Troglitazone	6.25 – 100

Compounds with the potential to cause cholestatic DILI exhibit cytotoxicity, determined by decreased ATP content and increased LDH leakage, in PHH when cultured in C-DILI™ sensitization media.

B-CLEAR® Assay: HuH-7 cells cultured for four weeks and modified with 1 µM dexamethasone and Matrigel overlay were exposed to fialuridine (100 µM), pioglitazone (100 µM), ritonavir (25 µM), and troglitazone (75 µM) for 24 h.

After drug treatment the cells were washed with Ca^{2+}-containing Hanks' Balanced Salt Solution (HBSS) (Ca^{2+} plus buffer), to maintain canalicular tight junctions, or Ca^{2+}-free HBSS (Ca^{2+} minus buffer), to disrupt tight junctions. Impaired bile acid transport was determined based on the cellular and biliary accumulation of [^{3}H]-taurocholate (200 nCi/mL) at a dosing concentration of 2 µM. The biliary excretion index (BEI) was determined as follows [4]:

$$\mathrm{BEI} = \frac{\mathrm{Total\ Accumulation}_{\mathrm{Plus\ Buffer}} - \mathrm{Cellular\ Accumulation}_{\mathrm{Minus\ Buffer}}}{\mathrm{Total\ Accumulation}_{\mathrm{Plus\ Buffer}}} * 100\%$$

Metabolomics and Proteomics Analysis: A single timepoint extraction of metabolites and proteins from sandwich-cultured Transporter Certified™ PHH was taken after 24-h exposure to fialuridine (100 µM), pioglitazone (100 µM), ritonavir (25 µM), and troglitazone (12.5 µM).

Metabolites and proteins were analyzed using a Q-Exactive Orbitrap mass spectrometer (ThermoFisher Scientific). Metabolites were analyzed in an untargeted manner. Proteins were analyzed using a data-independent acquisition MS/MS methodology using MaxQuant and Spectronaut™.

Results

C-DILI™ Assay using Transporter Certified™ PHH

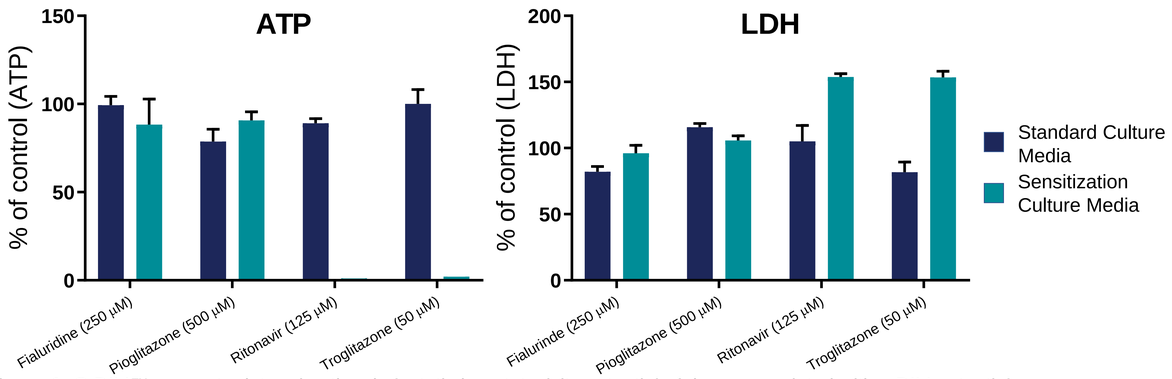
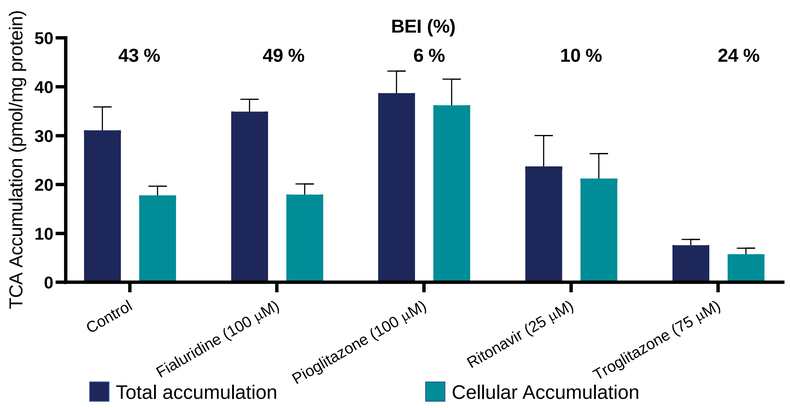


Figure 1: C-DILI™ assay to determine the cholestatic hepatotoxicity potential of drugs associated with a DILI potential

Ritonavir and troglitazone demonstrated a significant potential while fialuridine and pioglitazone showed no potential for cholestatic hepatotoxicity.

B-CLEAR® Assay using modified HuH-7 cell culture



Pioglitazone, ritonavir, and troglitazone impaired biliary transport of taurocholate based on the B-CLEAR® assay (n=3) after a 24-h exposure. Taurocholate BEI decreased for pioglitazone, ritonavir, and troglitazone exposures from control values of 43% to 6%, 10%, and 24%, respectively.

Figure 2: B-CLEAR® assay to determine impact of drugs with a DILI potential on taurocholate transport

Metabolomics and Proteomics using PHH

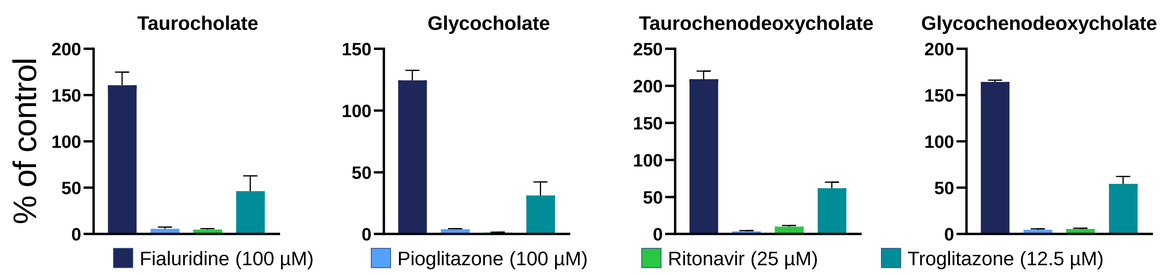


Figure 3: Metabolomics analysis using Ultra high resolution mass spectrometry to determine the effect drugs associated with a DILI potential have on the abundance of endogenous bile acids. Data shown is for total cell lysate.

Pioglitazone, ritonavir, and troglitazone reduced the cellular abundance (expressed as % of control; n=4) of taurocholate (4.8-46%), glycocholate (1.3-31%), taurochenodeoxycholate (3.5-9.8%), and glycochenodeoxycholate (4.5-5.3%); the cellular abundance of chenodeoxycholate, taurine, and glycine were unaffected.

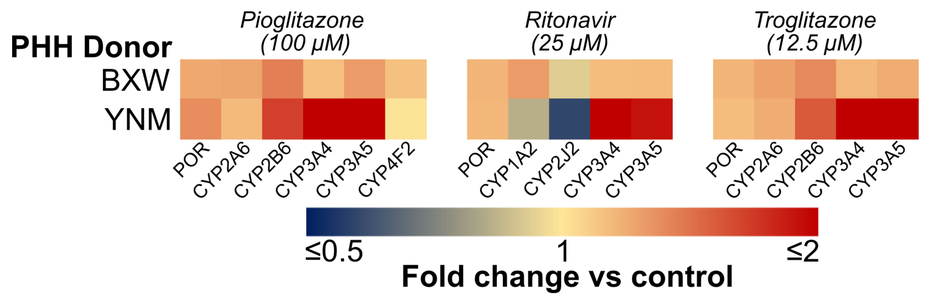


Figure 4: Proteomics heatmap showing changes in the abundance of Phase I enzymes induced by treatment with drugs associated with a DILI potential.

Proteomic analysis of PHH whole cell lysates (n=4) following a 24-h exposure to pioglitazone, ritonavir, and troglitazone revealed an increase in metabolic enzymes, including cytochrome P450 (CYP) 3A4, CYP3A5 and CYP reductase (POR).

Conclusion

Drugs such as pioglitazone, ritonavir, and troglitazone may initially impair bile acid transport, and induce an adaptive response that includes increased phase I metabolism and decreased cellular abundance of bile acids. However, the net effect of a drug on multiple hepatic pathways is necessary to determine the potential to cause cholestatic DILI. The C-DILI™ assay takes into consideration the adaptive response of hepatocytes when determining the potential of a drug to cause cholestatic DILI.

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References

1. Hayashi PH. *et al*. *Hepatology* (2017)
2. Licata A. *et al*. *Eur Rev Med Pharmacol Sci* (2017)
3. Kang HE. *et al*. *Drug Metab Dispos* (2019)
4. Liu X. *et al. Am J Physiol Gastrointest Liver Physiol (1999)*