# Prediction of Cholestatic Hepatotoxicity: Role of Transporter Regulation and Adaptive Response

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Qualyst Transporter Solutions
*In Vitro* Hepatic Models

## INTRODUCTION

Impaired canalicular efflux of bile acids has been postulated to play a role in drug-induced liver injury (DILI). Cholestatic DILI potential in humans has been associated with bile salt export pump (BSEP) inhibition; however, *in vitro* BSEP inhibition potency determinations do not correlate with *in vivo* cholestatic DILI severity. *In vivo* concentrations of bile acids are tightly regulated through synthesis, metabolism and transport mechanisms. Past studies have focused on acute interactions without evaluating the potential for adaptive responses (chronic interactions) of the hepatocyte. Chenodeoxycholic acid (CDCA) was used as a model bile acid (BA) to evaluate the concentration (72 hour exposure) and time course effects of chronic BA exposure on BA disposition in Transporter Certified™ sandwich-cultured human hepatocytes (SCHH). B-CLEAR® technology was used to assess the hepatobiliary disposition of $d_8$-TCA, $d_5$-GCDCA (endogenously generated), and the intracellular total bile acid (endogenously generated) pool in SCHH following 1, 3, 6, 12, 24, 48 and 72 hours exposure to 100 µM CDCA or $d_5$-CDCA. The mRNA content of key regulatory factors, synthetic enzymes, and transport proteins for BA was determined.

## METHODS

**Human Hepatocytes** Cryopreserved, Transporter Certified™ human hepatocytes in a sandwich configuration (24-well format) were cultured using Qual-Gro™ Induction Media; experiments were performed on Day 5 of culture.

**Treatments** Hepatocyte cultures were exposed to 100 µM CDCA or $d_5$-CDCA in culture media continuously for 1, 3, 6, 12, 24, 48 or 72 hours. Treatment with DMSO (0.1%) 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 0.1% DMSO 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.



## RESULTS AND DISCUSSION

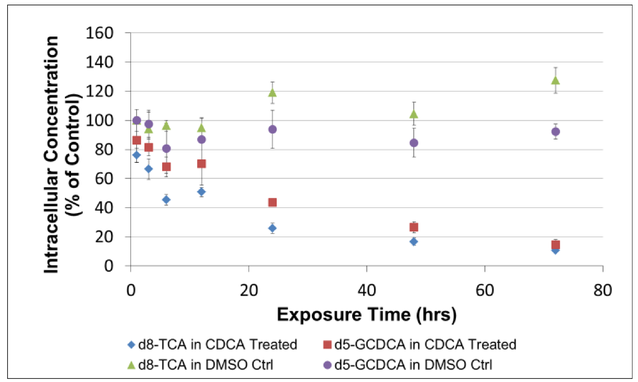



**Figure 1.** Intracellular concentration of $d_8$-TCA (exogenous) and $d_5$-GCDCA (endogenously generated) following timed continuous exposures to 100 µM CDCA or $d_5$-CDCA.

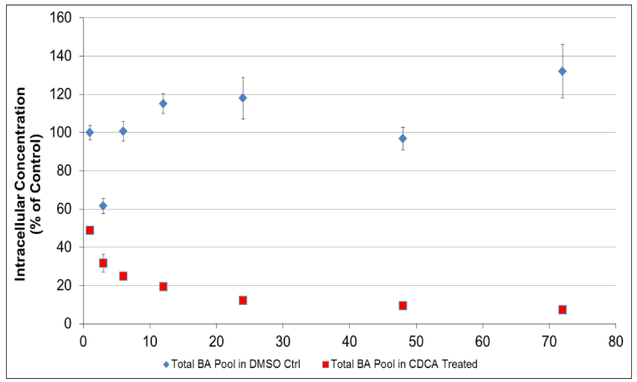
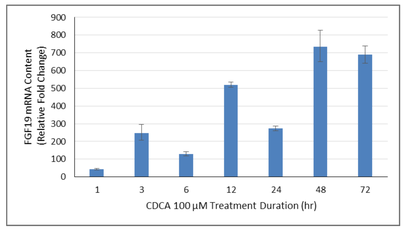
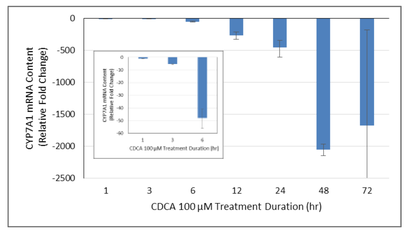
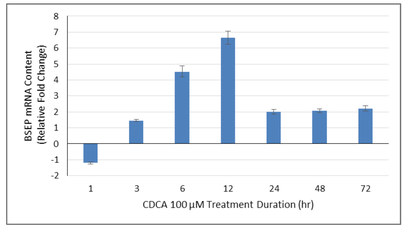
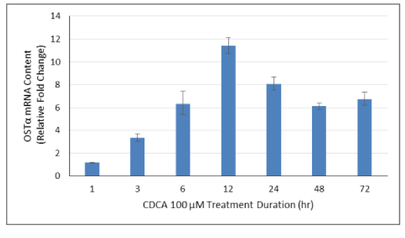



**Figure 2.** Total endogenous bile acid pool (CA, CDCA, TCA, GCA, TCDCA, GCDCA) over time following incubation with 0.1% DMSO or 100 µM $d_5$-CDCA.



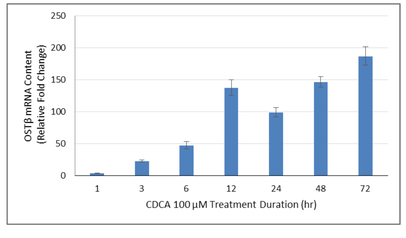
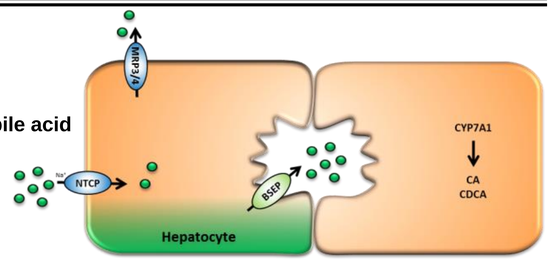



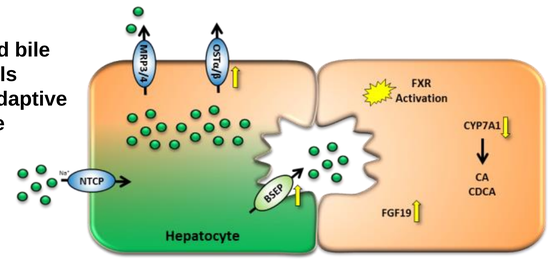

**Figure 3.** Changes in mRNA expression for selected transporters, synthetic enzymes, and regulatory factors following timed continuous exposure to 100 µM CDCA. No change (</> 2 fold of control) was observed for BCRP, CYP8B1, MRP2, MRP3, MRP4, NTCP, OATP1B3, OATP2B1, and PGP.

**Figure 4.**

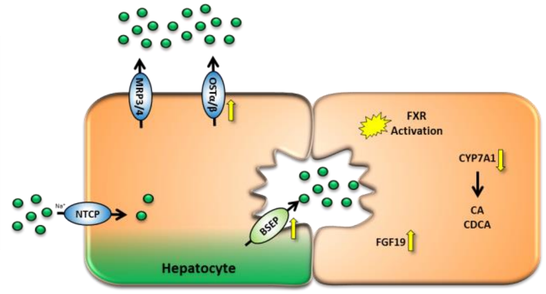
**Normal bile acid levels**




**Increased bile acid levels trigger adaptive response**




**Adaptive response: Induction of OSTα/β and BSEP along with suppression of CYP7A1 – leads to an increase in basolateral and canalicular efflux, and decreased intracellular concentration to prevent of cholestatic hepatotoxicity**




## CONCLUSIONS

- Adaptive responses to increased intracellular concentrations of bile acids occurred as early as 3 hours, and were maximal by approximately 24 hours.
- In the presence of 100 µM CDCA, the ICC of both $d_8$-TCA and $d_5$-GCDCA were significantly reduced at each exposure time, to 10.7% of control and 14.4% of control, respectively, after 72 hours of exposure (Figure 1).
- The ICC of the total bile acid pool was significantly reduced to < 10% of solvent control following exposure to CDCA (Figure 2).
- Exposure to CDCA decreased the CYP7A1 mRNA, and increased the mRNA content of BSEP and OSTα/β (Figure 3).
- The adaptive response (decreased synthesis and increased bile acid efflux by OSTα/β and BSEP), decreased the intracellular concentration of bile acids which decreases the potential for hepatotoxicity (Figure 4).

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