

Transporter Induction: A Potentially Important Pathway for the Prevention of Cholestatic Hepatotoxicity

Jonathan P. Jackson¹, Kimberly M. Freeman¹, Weslyn W. Friley¹, Robert L. St. Claire III¹, Jeffrey Edwards², and Kenneth R. Brouwer¹



¹ Qualyst Transporter Solutions, 2810 Meridian Parkway, Suite 100, Durham, NC 27713; ² Intercept Pharmaceuticals Inc, 4760 Eastgate Mall, San Diego, CA 92121

INTRODUCTION

In vivo, concentrations of bile acids (BA) are tightly regulated through synthesis, metabolism and transport mechanisms. Impaired canalicular BA efflux 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 have not been demonstrated to strongly correlate with *in vivo* cholestatic DILI severity. A potential reason for the lack of correlation is that past studies have focused on acute interactions and failed to evaluate the hepatocyte adaptive response. Bile acid concentrations in the hepatocyte are regulated by the Farnesoid X Receptor (FXR). Regulation of bile acid synthesis by FXR has been well defined; however, bile acid transport regulation has not. Transporter Certified™ sandwich-cultured hepatocytes from three human donors and B-CLEAR® technology were used to evaluate changes in the hepatobiliary disposition of a model bile acid, d₈-TCA following exposure to the FXR activators, obeticholic acid (OCA) or chenodeoxycholic acid (CDCA). The mRNA content of multiple uptake and efflux transporters was evaluated using TaqMan qRT-PCR. The effect of FXR activators on endogenous bile acids was also evaluated.

METHODS

Human Hepatocyte Sandwich-Culture Transporter Certified™, cryopreserved human hepatocytes from 3 donors (Triangle Research Labs, Xenotech) were cultured in 24-well BioCoat™ plates and overlaid with Matrigel™ 24 hours post-seeding. Qual-Gro™ induction culture medium was changed daily; studies were performed on Day 5.

Gene Expression Starting on Day 2, hepatocytes were exposed to OCA (0.003, 0.01, 0.1, 0.3, 1.0, 3.0 μM) or CDCA (0.1, 0.3, 1, 3.16, 10, 31.6, and 100 μM) in culture media for 72 hours. 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 Starting on Day 2, hepatocytes were exposed to OCA (1.0 μM) or CDCA (100 μM) in culture media for 72 hours. LCMS was used to quantitate endogenously generated cholic acid (CA), CDCA, and their taurine and glycine conjugates in cells, bile and cell culture media (CCM).

Hepatobiliary Disposition of d₈-TCA Starting on Day 2, hepatocytes were exposed to OCA (1.0 μM) or CDCA (100 μM) in culture media for 72 hours. Analysis of d₈-TCA was by LC-MS/MS which employed reversed-phase HPLC and electrospray ionization. B-CLEAR® technology was used to evaluate the hepatobiliary disposition of d₈-TCA following treatment.

RESULTS AND DISCUSSION

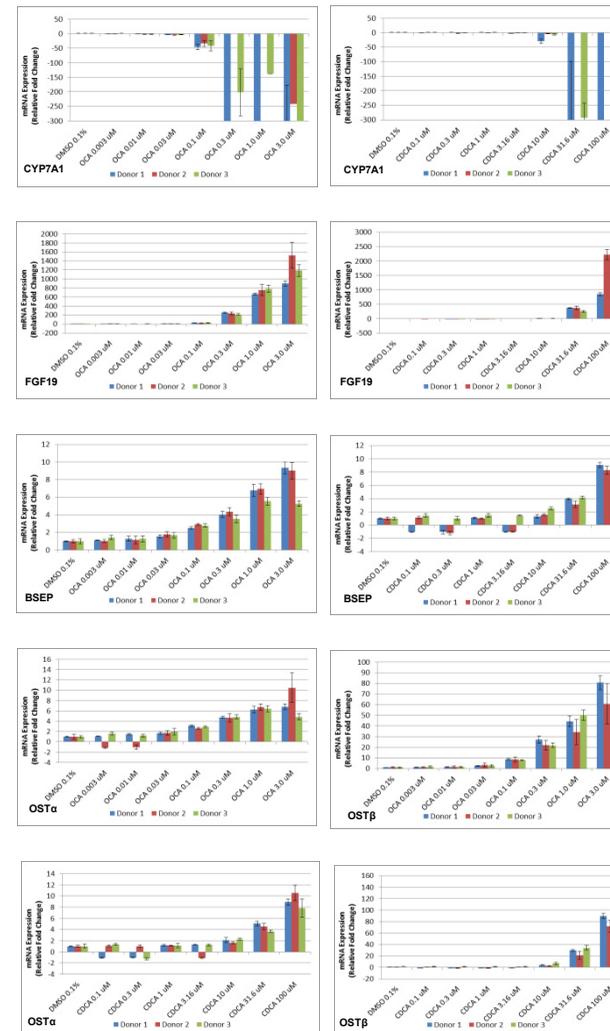


Figure 2. Effect of increasing concentrations of OCA or CDCA on the gene expression of bile acid synthetic enzymes, regulatory factors, and canalicular and basolateral efflux transporter proteins. Data represent mean ± SD of 3 replicates from 3 Transporter Certified™ human donors.

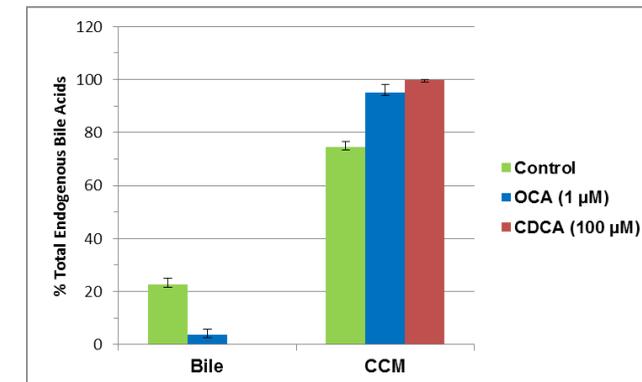
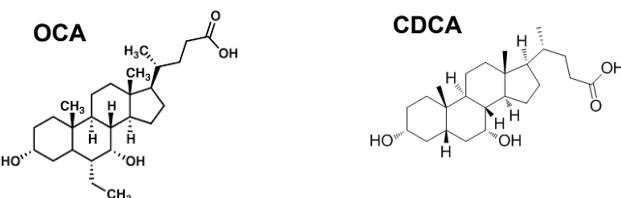


Figure 3. Effect of increasing concentrations of OCA or CDCA exposure on the disposition (bile or cell culture media) of endogenous bile acids. Data represent mean ± SD of 3 replicates from 3 Transporter Certified™ human donors.

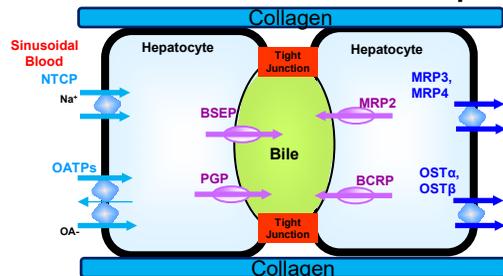
CONCLUSIONS

- Following 72 hrs of exposure to obeticholic acid (OCA) or chenodeoxycholic acid (CDCA), the biliary clearance of d₈-TCA was reduced to 40-57% and 19-28% of control, respectively.
- mRNA content of CYP7A1 was reduced to undetectable levels following both treatments. No marked changes were observed in mRNA content of the efflux transporters including PGP, MRP2, MRP3, MRP4, and BCRP following either treatment.
- OCA and CDCA treatment decreased the total, cellular and bile accumulation, biliary clearance and Kp ratio of d₈-TCA.
- Induction of FGF19 and suppression of CYP7A1 was observed for both OCA and CDCA treatments.
- Induction of BSEP was observed following exposure to OCA or CDCA, and ranged from 5.3 to 9.3-fold and 8.2 to 9.3-fold above solvent controls, respectively.
- Induction of basolateral efflux transporters OSTα (OCA: 7.4-fold, CDCA: 9.1-fold) and OSTβ (OCA: 42.9-fold, CDCA: 93.6-fold) mRNA content was observed and correlated with an increase in the media concentrations of endogenous bile acids.
- Induction of basolateral efflux of BA via OSTα/β may be an important compensatory pathway to prevent cholestatic hepatotoxicity when BSEP is inhibited.

Contact information: kennethbrouwer@qualyst.com
919-593-2519 (cell); 919-313-0163 (fax)



B-CLEAR® Sandwich-Cultured Hepatocytes



B-CLEAR® technology is covered by US Pat. No. 6,780,580 and other US and International patents both issued and pending.

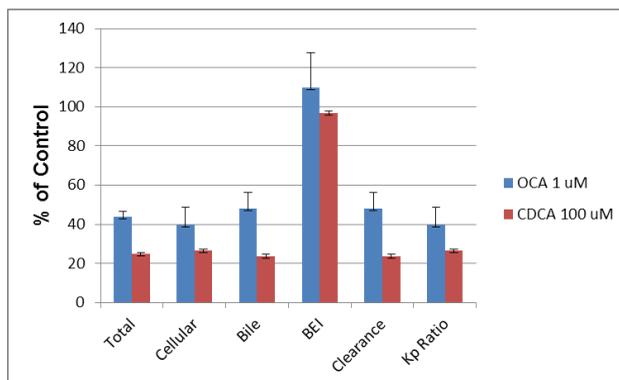


Figure 1. Hepatobiliary disposition of d₈-TCA following treatment with 1 μM OCA or 100 μM CDCA for 72 hours compared to DMSO control.