Basolateral Efflux Transporters: A Potentially Important Pathway for the Prevention of Cholestatic Hepatotoxicity

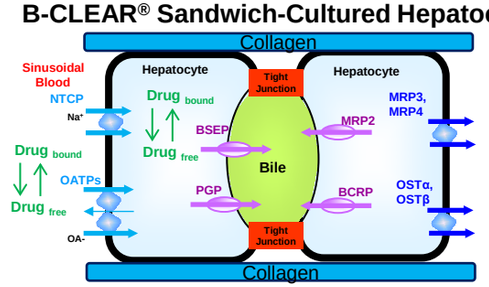
Jonathan P. Jackson[1], Kimberly M. Freeman[1], Weslyn W. Friley[1], Robert L. St. Claire III[1], Jeffrey Edwards[2], and Kenneth R. Brouwer[1]

[1] Qualyst Transporter Solutions, 2810 Meridian Parkway, Suite 100, Durham, NC 27713; [2] Intercept Pharmaceuticals Inc, 4350 La Jolla Village Drive, San Diego, CA 92122

INTRODUCTION

In vivo, concentrations of bile acids are tightly regulated through synthesis, metabolism and transport mechanisms. Many studies have identified the potential of compounds to alter the hepatobiliary disposition of bile acids through inhibition of their hepatic uptake and/or efflux. Bile acid homeostasis in the liver is regulated through various pathways including multiple transport proteins that remove bile acids from the blood and excrete them either back into the blood via basolateral efflux transporters or into the bile by canalicular efflux transporters. Most drugs that inhibit the efflux of bile acids can also inhibit their uptake to some extent. The relative extent of inhibition of *both* uptake and efflux (basolateral and canalicular) determines the net effect on the biliary clearance and intracellular accumulation of bile acids. A potential inhibitor's intracellular concentration is also important since it determines the extent of transport inhibition and drives toxicity likely by elevating intracellular concentrations of bile acids. Regulation of bile acid synthesis and its role in bile acid homeostasis has been well defined; however, bile acid transport has not. We evaluated the hepatobiliary disposition of a model bile acid d_8-taurocholate (d_8-TCA) and expression of bile acid synthetic enzymes, transport proteins and regulatory factors following chronic exposure to chenodeoxycholic acid (CDCA) to determine the role of bile acid transport (uptake & efflux) in bile acid homeostasis..

B-CLEAR® Sandwich-Cultured Hepatocytes



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

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.

Treatment of SCHH. Starting on Day 2, hepatocytes were exposed to CDCA in culture media at 0.1, 0.3, 1, 3.16, 10, 31.6, and 100 µM for 72 hours. Dosing solutions were made fresh daily in Qual-Gro™ induction culture medium.

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.

Hepatobiliary Disposition of d_8-TCA. Using B-CLEAR® technology, the effects of 100 µM CDCA on the hepatobiliary disposition of d_8-TCA and endogenous bile acids were determined by LC-MS/MS which employed reversed-phase HPLC and electrospray ionization.

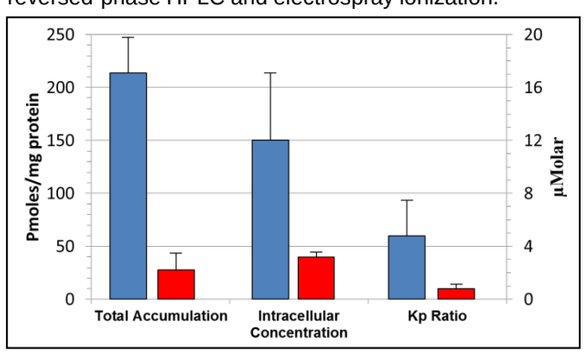
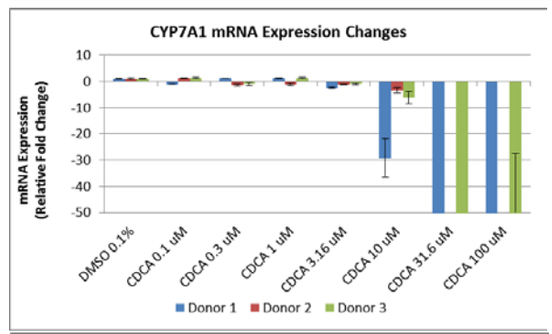
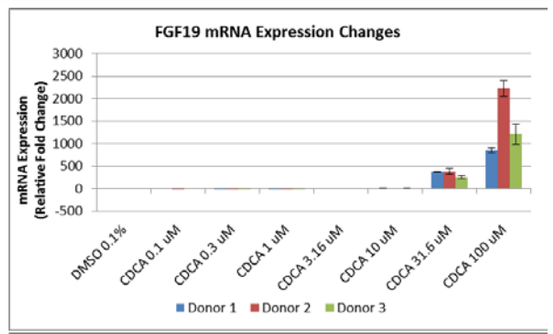
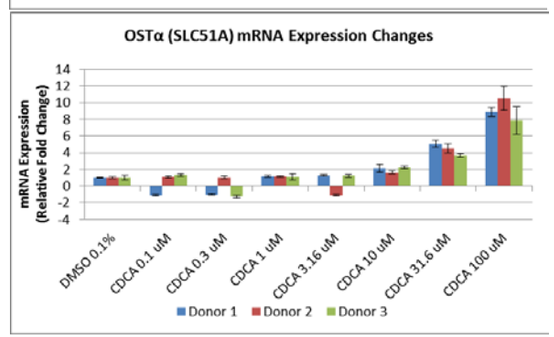


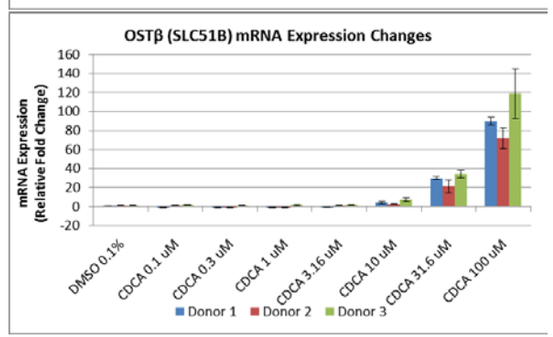
Figure 1. Hepatobiliary disposition of d_8-TCA following treatment with 100 µM CDCA for 72 hours (■) compared to DMSO control. (■)

RESULTS AND DISCUSSION









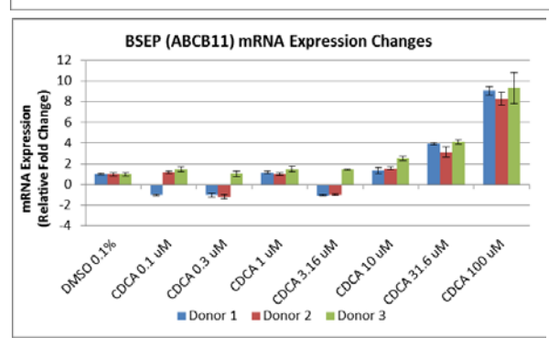


Figure 2. Effect of increasing concentrations of CDCA on expression of bile acid synthetic enzymes, transporter proteins and regulatory factors. Data represent mean ± SD of 3 replicates from 3 donors.

CONCLUSIONS

- Total accumulation, intracellular concentration, and Kp ratio for d_8-TCA were all decreased following chronic exposure to CDCA.
- CDCA exposure increased the expression of the nuclear regulator factors FGF19 (1000X) and SHP (4X), decreased expression of CYP7A1(> 200X), and increased expression of the basolateral (OSTα/β – 10X, 100X) and canalicular (BSEP – 5X) transporters for bile acids.
- Decreased expression of CYP7A1 resulted in a decrease in synthesis of endogenous bile acids (TCA, GCA) to 25 % of control.
- Induction of OSTα/β increased media concentrations of endogenously generated TCA and GCA to 115 % of control.
- Inhibition interactions with basolateral and canalicular transporters in the induced state may be a critical parameter in the development of cholestatic hepatotoxicity.