Intracellular Concentrations of Endogenous Bile Acids in Transporter Certified™ Sandwich-Cultured Hepatocytes: Relevance for Prediction of Cholestasis.

Qualyst Transporter Solutions, LLC

Jonathan P. Jackson, Kimberly M. Freeman, Weslyn W. Friley, Robert L. St. Claire III, and Kenneth R. Brouwer

Qualyst Transporter Solutions, 2810 Meridian Parkway, Suite 100, Durham, NC 27713

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). Many compounds that have been shown to inhibit BSEP mediated efflux of bile acids also inhibit their uptake into hepatocytes. Inhibition studies in whole cell models using exogenously administered bile acids can be confounded if the compound significantly inhibits the uptake of bile acids. Cyclosporine A (CsA) inhibits both the uptake and efflux of bile acids. Analysis of endogenously generated bile acids may allow for separation of the uptake and efflux effects in interaction studies. Transporter Certified™ sandwich-cultured hepatocytes and B-CLEAR® technology were used to evaluate changes in the hepatobiliary disposition of endogenous bile acids following treatment with 10 µM CsA for 10 minutes to 12 hours. Treatment with d_5-chenodeoxycholic acid (CDCA) was used to understand the impact of FXR activation on endogenous bile acid disposition.

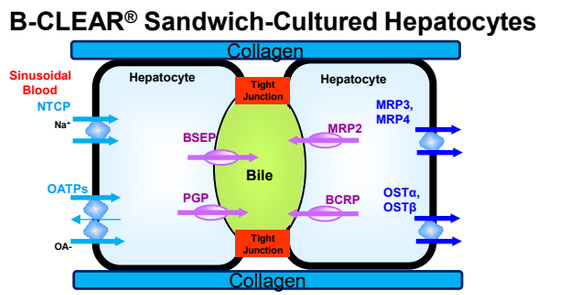
METHODS

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

Treatments Hepatocyte cultures were exposed to CSA (10 µM) or d_5-CDCA (100 µM) as the positive control in culture media for various time points from 10 minutes to 12 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 (CCM).



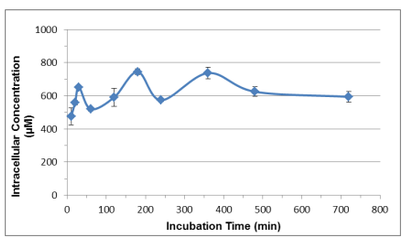


Figure 1. Intracellular concentration of CSA over time following incubation with 10 µM CSA.

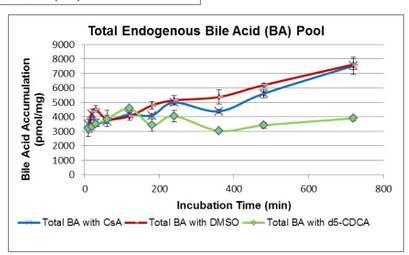


Figure 2. Total endogenous bile acid pool over time following incubation with 10 µM CSA, 0.1% DMSO, or 100 µM d_5-CDCA.

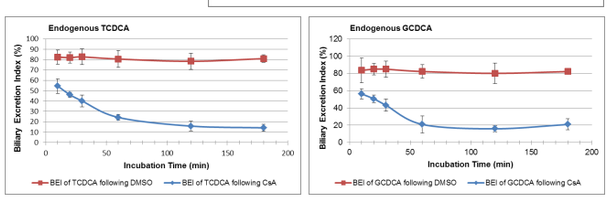


Figure 3. Biliary Excretion Index (BEI) for the two major endogenous bile acids (TCDCA and GCDCA) over time following incubation with 0.1% DMSO or 10 µM CsA.

RESULTS AND DISCUSSION

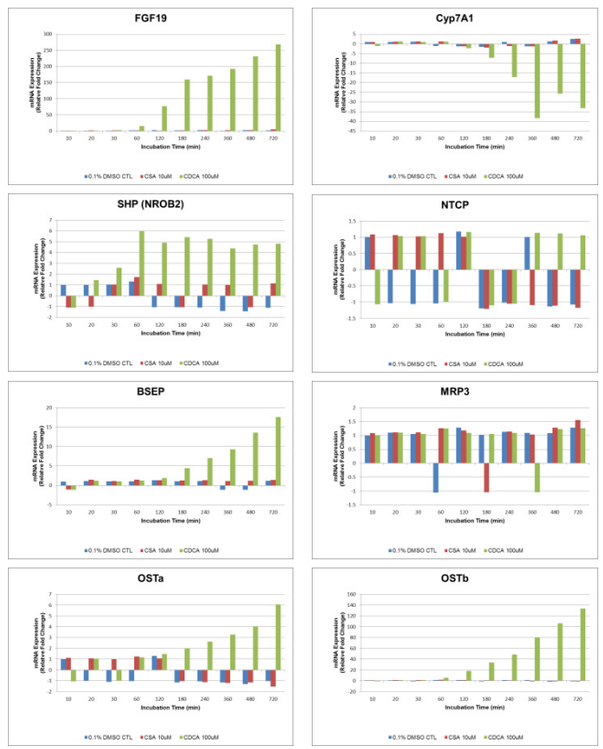


Figure 4. Effect of increasing incubation times of CSA (10 µM) or CDCA (100 µM) on the gene expression of regulatory factors, bile acid synthetic enzymes, and canalicular and basolateral transporter proteins

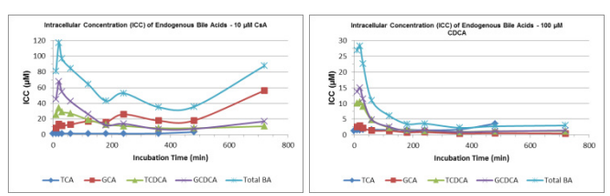


Figure 5. Effect of increasing incubation times of CSA (10 µM) or CDCA (100 µM) on the intracellular concentration of endogenously generated bile acids.

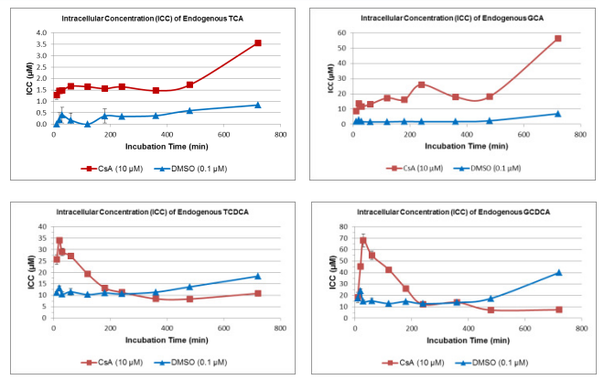


Figure 6. Intracellular concentration of the four major endogenous bile acids (TCA, GCA, TCDCA and GCDCA) over time following incubation with 0.1% DMSO or 10 µM CsA.

CONCLUSIONS

- Intracellular concentrations of CsA were relatively constant over 12 hours (Figure 1).
- The total endogenous bile acid pool (cell + bile + media) increased over time in the DMSO control. CsA had no effect on the total endogenous bile acid pool, whereas exposure to d_5-CDCA decreased the total endogenous bile acid pool (Figure 2).
- Consistent with inhibition of BSEP by CsA, the biliary excretion index (BEI) for all bile acids decreased over time (Figure 3).
- No changes in gene expression were detected following exposure to CsA. Exposure to d_5-CDCA increased mRNA expression of FGF19, SHP, BSEP, and OSTα/β, and decreased the mRNA for Cyp7A1 (Figure 4).
- The intracellular concentration of total endogenous bile acids was increased in the presence of CsA (Figure 5).
- In the presence of CsA, the intracellular concentrations of TCDCA and GCDCA decreased, and the Intracellular concentrations of TCA and GCA increased over time (Figure 6) .
- The decrease in the intracellular concentration of TCDCA and GCDCA and the lack of change in total endogenous bile acids is consistent with their conversion to TCA and GCA.
- Endogenous bile acid concentrations may provide a sensitive tool to evaluate the intracellular effects of compounds that alter the hepatobiliary disposition of bile acids.

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